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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

Clinical Pharmacologic Effects of Thymidine Plus 5-FU[1,2]

Takao Ohnuma,* John Roboz, Samuel Waxman, Eugene Mandel, Daniel S. Martin, and James F. Holland[3]

SUMMARY

Ten patients with advanced cancer were given 24 courses of thymidine (TdR) plus 5-FU. In the initial part of the study, TdR was given by continuous infusion at a dose of 8 g/m²/24 hrs × 5 days, and 5-FU was given as an iv bolus daily × 5. During the continuous TdR infusion, TdR serum levels ranged from 10^{-6} to 10^{-5} M, and thymine serum levels ranged from 10^{-5} to 10^{-4} M. 5-FU serum decay on Day 1 was rapid, and serum decay on Day 5 was slightly prolonged in two of three patients studied. The optimal dose of 5-FU with continuous infusion of TdR seemed to be between 200 and 400 mg/m² daily × 5 in heavily pretreated patients. In the second part of the study, rapid infusion of TdR at a dose of 8 g/m²/2½ hrs followed by 5-FU resulted in markedly prolonged 5-FU serum decay (initial serum half-life, ~ 240 mins) and definite increase in clinical toxicity. The dose-limiting toxic effect of both schedules was myelosuppression. Administration of TdR in one tenth of the initial infusion time resulted in a nearly 100-fold increase in peak TdR levels. Thymine was the major metabolite of TdR. 5-FU did not influence TdR blood levels. The protracted 5-FU serum decay appears to account at least in part for the increased toxicity of 5-FU when administered following TdR.

[Cancer Treat Rep 64:1169–1177, 1980]

5-FU is one of the most widely used antineoplastic agents in man. It is the reference agent in the treatment of adenocarcinoma of the colon and rectum and is used in combination with other agents for the treatment of breast and gynecologic cancers. Martin et al (1,2) observed that administration of thymidine (TdR) prior to 5-FU resulted in striking augmentation of the antitumor effect of 5-FU against advanced $CD8F_1$ murine mammary tumor. They correlated the improved antitumor effects with enhanced incorporation of 5-FU into RNA (2,3).

Based on these animal data, initial clinical studies of the TdR and 5-FU combination have been done at various institutions (4–7). This paper describes our study of the pharmacologic effects of TdR plus 5-FU in patients with advanced cancer.

MATERIALS AND METHODS

Ten patients (seven men and three women; median age, 56 years [range, 28–64]) with advanced cancer (colon [four patients] and rectum, kidney, renal pelvis, lung, breast, and adenocarcinoma of unknown primary [one each]) received a total of 24 courses of treatment. All but one patient (adenocarcinoma of unknown primary) had received prior chemotherapy and all six patients with colorectal and breast carcinoma had received 5-FU (Roche Laboratories, Nutley, NJ). Their performance status (Cancer and Leukemia Group B criteria) were

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[2]Supported in part by contract N01-CM-53837 (Division of Cancer Treatment) and Public Health Service grant CA-15936 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Chemotherapy Foundation, Inc, New York, NY.

[3]Departments of Neoplastic Diseases and Medicine (Oncology), Mt. Sinai School of Medicine of the City University of New York, and Department of Surgery, Catholic Medical Center, Woodhaven, NY.

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1+ (ambulatory; four patients), 2+ (< 50% of time in bed; two), 3+ (> 50% of time in bed; three), and 4+ (100% bedridden; one). All patients gave informed consent prior to treatment.

The initial protocol (Protocol I) consisted of continuous TdR (15 g/500-ml bottle, formulated by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) infusion at a dose of 8 g/m²/24 hrs for 5 days and 5-FU at a dose of 100 mg/m² by iv bolus daily (first dose 2 hrs after the start of TdR infusion) for 5 days. In the absence of dose-limiting toxicity, the 5-FU dose was escalated in subsequent patients to 200 mg/m²/day × 5 and then to 400 mg/m²/day × 5. The TdR dose schedule was adopted from clinical data for methotrexate plus TdR (8) and the 5-FU schedule was the standard loading course (9,10).

The dose schedule was modified in the second protocol (Protocol II). On Day 1, TdR was administered alone at a dose of 8 g/m²/2½ hrs; on Day 3, 5-FU was given as an iv bolus at the end of the TdR infusion of 8 g/m²/2½ hrs; and on Day 5, 5-FU was given alone as an iv bolus. The second protocol was constructed so that pharmacologic effects of TdR on 5-FU could be compared with those of TdR alone and 5-FU alone with the then available supply of TdR. In the last phase of the study, the patients received TdR (8 g/m²/2½ hrs) followed by 5-FU (400 mg/m², iv bolus) at 4- or 5-week intervals (Protocol IIb).

At the initiation of treatment all patients had wbc counts of > 4000/μl, platelet counts of > 100,000/μl, and creatinine levels of < 1.5 mg/dl. All but one patient had normal hepatic function (patient 7 had a bilirubin of 1.9 mg/dl, an SGOT of 87 milliunits/ml, and an SGPT of 64 milliunits/ml. Therapeutic effects of the treatment were evaluated according to our current response criteria (11).

Serum 5-FU, 5-fluorodeoxyuridine, TdR, and thymine (T) levels were determined in the same sample by selected ion monitoring (4). Briefly, serum was deproteinized by molecular filtration (Amicon Corp, Lexington, MA; exclusion limit, 50 × 10^3 daltons), evaporated to dryness under nitrogen gas, and flash-methylated with trimethylaniliniumhydroxide (Pierce Chemical Co, Rockford, IL) at 270°C in the injector port of the gas chromatograph-mass spectrometer (Model 3300) - computer (Model 6000) system (Finnigan, Sunnyvale, CA). After passing through a 5-ft glass column filled with 10% SP-2100 (methyl silicone; Supelco, Bellefonte, PA), TdR, T, 5-FU, and 5-fluorodeoxyuridine were quantified in the same sample by monitoring their protonated molecular ions obtained by chemical ionization with isobutane (which also served as carrier gas). 5-Chlorodeoxyuridine and 5-chlorouracil served as the internal standards. The detection limit was 50 pg injected or 25 ng/ml of serum (10^{-7} M range), approximately the same for all four compounds. The patient's own serum served to correct for endogenous TdR present (~ 2 × 10^{-7} M).

Inhibitory effects of 5-FU in the presence or absence of TdR on human lymphoid cell lines were studied as previously described (12). For determination of the effects of TdR on 5-FU uptake into RNA, the cells were exposed to 5-fluoro[6-^{3}H]uracil (Amersham/Searle, Arlington Heights, IL) at 37°C in the presence of various concentrations of TdR. Cellular RNA was extracted (13) and the incorporation of radioactive 5-FU into RNA was measured in a liquid scintillation counter (LS-355; Beckman Instruments, Fullerton, CA).

RESULTS AND DISCUSSION

The clinical effects of TdR plus 5-FU given under Protocol I are summarized in table 1. The 5-FU iv bolus was escalated from a dose of 100 to 200 to 400 mg/m²/day for 5 days. At the highest dose studied, two of three patients developed severe leukopenia

TABLE 1.—Toxicity of TdR + 5-FU treatment (Protocol I)*

5-FU dose			No. of evaluable	Nadir wbc count (× 10^3/μl)				Nadir platelet count (× 10^3/μl)			Nausea and				
mg/m²/day × 5 days	mg/m²/course	Patient Nos.	courses/total No.	> 4	2–4	1–2	< 1	> 100	50–100	< 50	vomiting	Mucositis	Diarrhea	Skin eruption	Alopecia
100	500	1	1/1	1	0	0	0	1	0	0	0	0	1	0	0
200	1000	1, 2, 3	3/3	2	1	0	0	3	0	0	2	1	0	0	0
400	2000	1, 3, 4	3/3	1	0	0	2†	3	0	0	3	2	1	1	2
Totals			7/7	4	1	0	2	7	0	0	5	3	2	1	2

*Continuous iv TdR infusion at a dose of 8 g/m²/24 hrs × 5 days and 5-FU iv bolus daily × 5 days.
†Both patients developed pneumonia, were treated with cephalothin and gentamicin, and recovered.

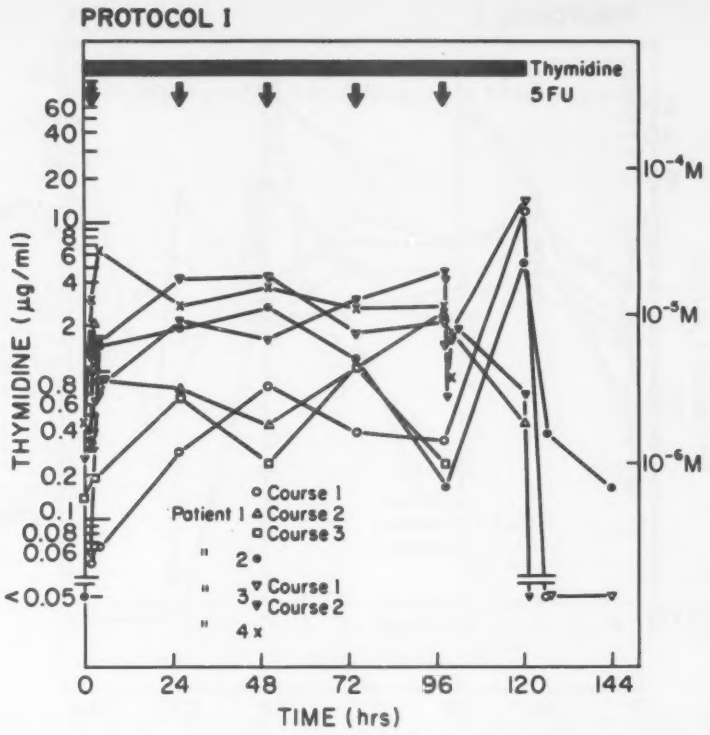

FIGURE 1.—Serum TdR levels in 4 patients during and after 7 courses of continuous iv infusion of TdR (8 g/m²/24 hrs) for 5 days and 5-FU iv daily × 5 (arrows).

(< 1000 cells/μl, nadir by Day 18–22; recovery to > 3000 cells/μl by Day 22–28) and mucositis. Both patients developed pneumonia and were treated iv with antibiotics (cephalothin and gentamicin) as inpatients and both recovered fully. It was concluded that the maximally tolerated dose had been reached. The optimal dose of 5-FU with continuous infusion of TdR seems to be between 200 mg and 400 mg/m²/day × 5. While the addition of TdR appeared to have augmented the 5-FU toxicity to some extent, it was nevertheless thought that the myelosuppression observed in this study might have been produced by loading courses of 5-FU alone (9,10) in patients who had already received maximal doses of myelosuppressive chemotherapy.

The TdR and T serum levels were measured during seven courses of continuous TdR infusion (8 g/m²/24 hrs × 5 days) in four patients, and the results are shown in figures 1 and 2. During TdR infusion, TdR serum levels were maintained in a range of 10^{-5} to 10^{-6} M and T levels were maintained in a range of 10^{-4} to 10^{-5} M. The causes of widely fluctuating TdR levels, even in some patients to whom the drug was constantly administered with a McGaw infusion pump (McGaw Laboratories, Santa Ana, CA), were not clearly determined. 5-FU serum levels were measured in three patients on Days 1 and 5 (fig 3). Initial 5-FU serum half-lives on Day 1 were approximately 7, 10, and 30 mins, whereas those on Day 5 were 17, 70, and 30 mins. These data suggested a minor to moderate prolongation of 5-FU decay by Day 5. These results are consistent with those of Kirkwood et al (7) who described progressive prolongation of 5-FU serum half-lives during a course of five daily injections of the drug and further augmentation of 5-FU serum half-lives with infusions of TdR. We did not study the pharmacokinetics of 5-FU alone in this protocol, but the

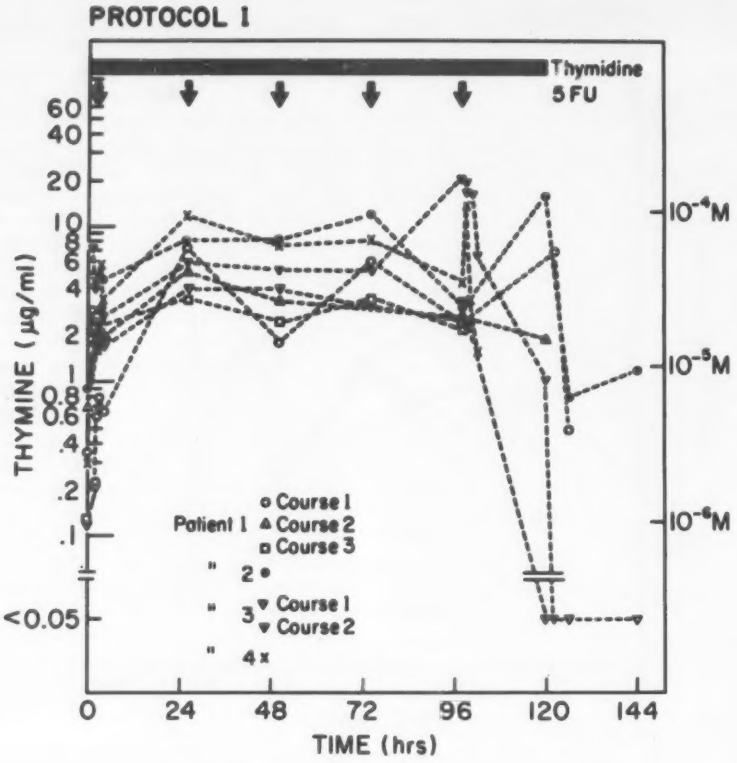

FIGURE 2.—Serum T levels in 4 patients during and after 7 courses of continuous iv infusion of TdR (8 g/m²/24 hrs) for 5 days and 5-FU iv daily × 5 (arrows).

serum half-lives of 5-FU on Day 1 together with TdR appeared similar to those without TdR (fig 4).

Concurrent investigation of 5-FU and TdR in human cell lines (MOLT 3, B46M, and RPMI-8422) in vitro revealed that TdR at concentrations of 10^{-5} and 10^{-6} M was unable to displace the 5-FU dose-cell growth inhibition curve, while 10^{-4} M TdR resulted in substantial augmentation of 5-FU uptake into RNA and displacement of the 5-FU dose-cell growth inhibition curve (Ohnuma T, unpublished observation).

The clinical pharmacologic data from Protocol I as well as the in vitro data suggested that insufficient serum TdR levels were achieved by the dose schedule in Protocol I. The protocol was therefore modified to administer TdR in one tenth of the infusion time (Protocol II) in the hope of increasing a peak TdR concentration to approach 10^{-4} M, a biologically active concentration in vitro. Clinical data obtained from Protocol II are summarized in table 2. Although the total dose of 5-FU given per course was only two fifths that in Protocol I and only one of the two doses was accompanied by pretreatment with TdR, all three patients who received doses of 400 mg/m² of 5-FU developed hematologic toxicity (wbc count nadir on Day 10–24; recovery to > 3000/μl at Day 25–26). After receiving 400 mg/m² of 5-FU with TdR, one patient with pre-existing hepatic dysfunction developed severe mucositis, leukopenia of 250 cells/μl, and thrombocytopenia of 60,000 cells/μl.

In the latter phase of the study, three patients received ten courses of treatment with TdR (8 g/m²/2½ hrs) followed immediately by 5-FU (400 mg/m², iv bolus) every 4–5 weeks (Protocol IIb). The toxicity encountered is shown in the lower half of

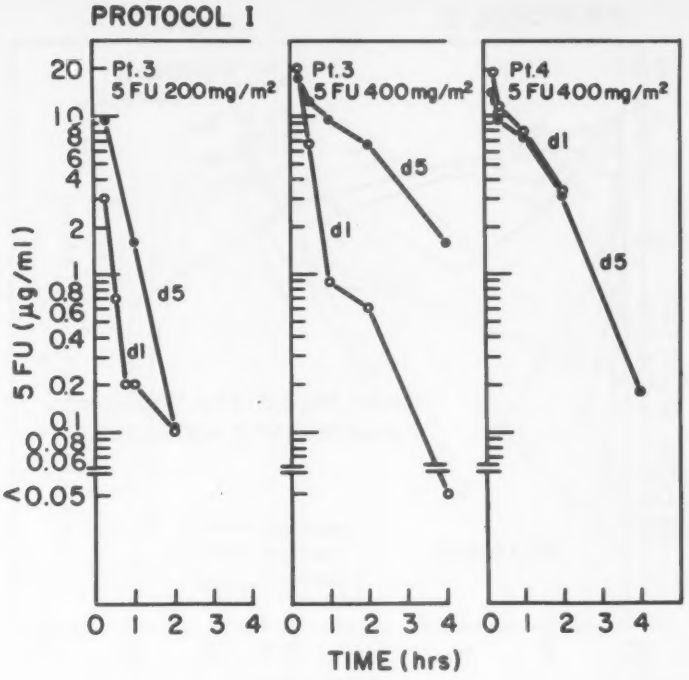

FIGURE 3.—Serum 5-FU decay after iv bolus of 5-FU given on Day 1 (open circles) and on Day 5 (solid circles) of TdR (8 g/m²/24 hrs) continuous infusion for 5 days.

TABLE 2.—Toxicity of TdR + 5-FU treatment (Protocol II)

5-FU Dose			No. of evaluable courses/ total No.	Nadir wbc count (× 10^3/μl)				Nadir platelet count (× 10^3/μl)			Nausea and vomiting	Muco-sitis	Diar-rhea	Skin eruption	Alope-cia
mg/m²/day	mg/m²/ course	Patient Nos.		> 4	2–4	1–2	< 1	> 100	50–100	< 50					
200*	400	1, 5, 6, 8	3/4	2	1	0	0	3	0	0	1	3	0	0	0
400*	800	5, 6, 7	3/3	0	2	0	1†	2	1†	0	1	2	2	0	1
400‡	400	4, 9, 10	10/10	5	2	3§	0	9	1	0	1	0	0	5	1
Totals			16/17	7	5	3	1	14	2	0	3	5	2	5	2

*TdR (8 g/m²/2½ hrs) on Day 1, TdR (8 g/m²/2½ hrs) then 5-FU iv bolus on Day 3 and 5-FU iv bolus on Day 5.
†1 patient (No. 7) had pre-existing hepatic dysfunction and severe hematologic toxicity.
‡Patients in the latter phase of the study received TdR (8 g/m²/2½ hrs) then 5-FU (400 mg) iv bolus every 4–5 wks (Protocol IIb).
§2 of 3 courses were accompanied by sepsis.

table 2. One patient who had had minimal previous chemotherapy tolerated three courses of treatment without myelosuppression. The second patient, after treatment in Protocol I, received five courses in Protocol IIb, and two courses were accompanied by leukopenia of 2000 and 4000 cells/μl. The third patient had repeated leukopenia with the wbc count nadir between 1000 and 2000/μl after each of three courses of treatment. Sepsis occurred twice in the third patient. Other clinical toxic effects included

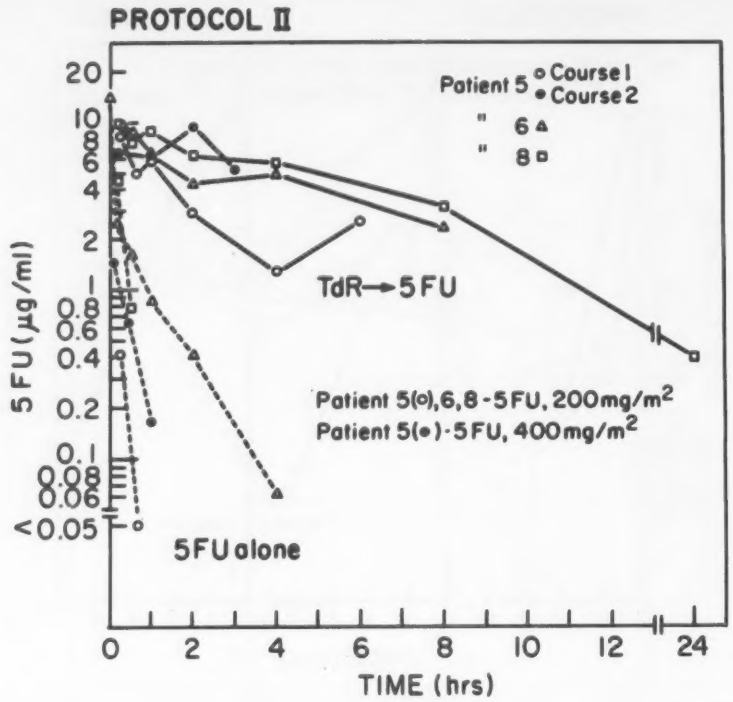

FIGURE 4.—Serum 5-FU levels after iv bolus of 5-FU alone (broken lines) and priming with TdR (8 g/m²/2½ hrs) (solid lines).

nausea and vomiting, mucositis, diarrhea, skin eruption, and alopecia, none was dose-limiting. Although the number of patients studied was small, there was no clear difference in parameters of clinical toxicity between the patients in Protocols I and II. This remarkable augmentation of 5-FU toxicity after a short infusion of TdR is similar to that reported by Woodcock et al (5) who gave TdR as a 30-min infusion followed by 7.5–10 mg/kg of 5-FU iv bolus.

Serum TdR and T levels following the 2½-hr infusion of TdR with or without 5-FU iv bolus are illustrated in figures 5 and 6. The peak TdR serum level achieved by this schedule approached 10^{-3} M. Serum T decay was in general more prolonged than that of TdR. 5-FU did not seem to have influenced the general shape of the TdR serum levels. In contrast, TdR pretreatment resulted in a marked delay in 5-FU serum clearance. As shown in figure 4, administration of 5-FU alone resulted in a rapid decrease in 5-FU level, while TdR infusion followed by 5-FU administration in four courses in three patients resulted in a striking delay in serum clearance of 5-FU (average initial serum half-life, approximately 240 mins). An apparent increase in clinical toxicity was at least partly correlated with a marked increase in 5-FU serum half-life.

None of the patients responded with > 50% regression of measurable tumor, but two patients (patient 4 with carcinoma of rectum treated according to Protocol I [5-FU at a dose of 400 mg/m²/day × 5] then Protocol IIb, and patient 7 with breast carcinoma treated according to Protocol IIb) had stable disease lasting 6 and 3 months, respectively.

Clinical studies established that the major pharmacologic effect of TdR was prolonged 5-FU serum decay. In addition, we observed enhanced 5-FU incorporation into RNA of human leukemic cells in

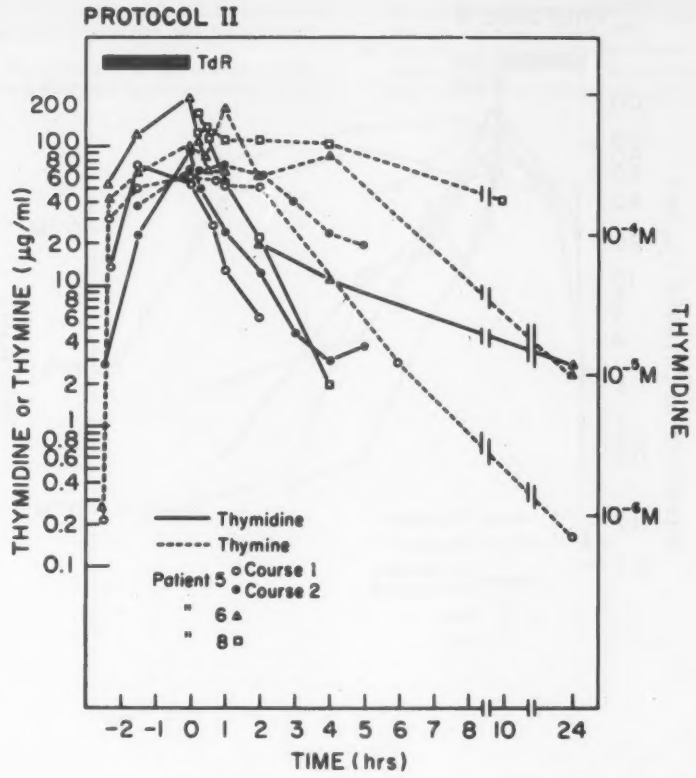

FIGURE 5.—Serum TdR and T levels during and after administration of TdR (8 g/m²/2½ hrs).

vitro in the presence of large concentrations of TdR. It is not clear, therefore, whether prolonged 5-FU decay or the enhanced 5-FU incorporation into RNA played a major role in the clinical observation of enhanced toxicity. Previous 5-FU infusion regimens produced dose-limiting stomatitis (14,15). Therefore, prolonged 5-FU serum decay alone in Protocol II did not explain the dose-limiting leukopenia. Indeed, TdR infusion given concurrently with 5-FU infusion changed the dose-limiting toxicity from gastrointestinal effects to myelosuppression (6).

Since both 5-FU and TdR are metabolized in the liver through the same enzyme system (16), prolonged 5-FU serum decay after rapid TdR infusion might have been caused by saturation of the catabolic pathway by TdR. This was best achieved by rapid TdR infusion in Protocol II. Prolonged TdR infusion in Protocol I appears to have produced a TdR serum level well below the liver's capacity to catabolize TdR. This difference may be reflected in the higher T level achieved in Protocol I and in the higher than expected peak serum TdR levels achieved in Protocol II.

The present study did not provide an optimal phase II dose. Nevertheless, available data from Protocol IIb suggest that an infusion of TdR at a dose of 8 g/m²/2½ hrs followed immediately by 5-FU at a dose of 400 mg/m² by iv bolus, repeated as soon as a patient recovers from hematologic toxic effects, may be tested in patients with minimal prior chemotherapy and no hepatic dysfunction. Whether TdR plus 5-FU produces better therapeutic effects than 5-FU alone in man remains to be evaluated.

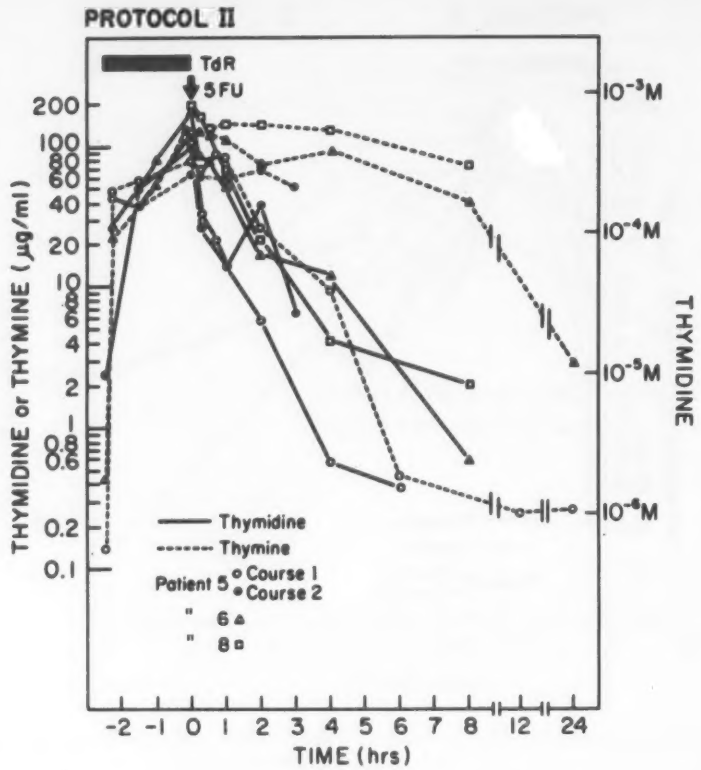

FIGURE 6.—Serum TdR and T levels during and after administration of TdR (8 g/m²/2½ hrs) followed immediately by iv bolus of 5-FU (arrow).

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Brain Tumor Chemotherapy: An Evaluation of Agents in Current Use for Phase II and III Trials[1,2]

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SUMMARY

The reported English-language literature since 1969 on chemotherapy for malignant brain tumors has been collated, and reports of drugs or drug combinations used in phase II or III trials or with adjunctive therapies are reviewed. A significant fraction of the literature contained only anecdotal information, and even in the reports of more systematic trials, the criteria used to evaluate response were variable, ranging from subjective evaluation of clinical improvement to more stringent evaluations using one or more neurodiagnostic tests. A more consistent method of determining response is needed to facilitate comparison of different treatment protocols in the future, and guidelines for this are suggested. It appears that drugs of small molecular size (< 450 daltons) and high lipid solubility have been the most effective. Single-agent chemotherapy using cell-cycle-specific drugs has, however, been disappointing. The nitrosoureas (especially BCNU) used either singly or in combination have consistently been the most effective agents in phase II and III trials. The combination of irradiation with adjunctive BCNU remains, for the present, the standard against which all other phase II and III protocols should be compared. New drugs for phase II evaluations are sorely needed. There is also need for new combination-drug and sequential-drug protocols for evaluation. The use of radiosensitizing agents with more novel radiation therapy fractionations and in combination with adjunctive drug therapies holds some promise for improving the disappointing results obtained thus far with chemotherapy for malignant brain tumors.

[Cancer Treat Rep 64:1179–1205, 1980]

The incidence of malignant primary intracranial neoplasms in the US is approximately 4.2/100,000, and that of metastatic tumors to the brain is 8.5/100,000 (1). Therefore, > 27,000 new cases of primary and secondary brain tumor will emerge in the US each year. In addition, the number of metastatic tumors appears to be increasing as a result of the rising incidence of lung cancer and the increased longevity of systemic tumor patients that has been achieved with current chemotherapeutic methods.

Malignant brain tumors are almost always fatal. Of 420 histologically verified cases, only 50 patients (11.9%) survived 12 months, and only 16 (3.7%) were alive at 24 months (2). Rarely are patients with malignant brain tumors cured by surgery and/or radiation therapy. The median survival time following surgery and radiation therapy in most reported series has remained at the 7.5–9.5 months reported by several investigators, including Jelsma and Bucy (3–5) in the review of their experience with glioblastoma multiforme. For metastatic tu-

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mors, the prognosis is even more pessimistic, with a median survival time of 3–4 months after radiation therapy (6).

Shapiro and Ausman's (7) excellent review of clinical and experimental trials updates to 1969 the world literature in English on all drugs used to treat malignant brain tumors. As a significant portion of this literature constitutes merely anecdotal reports, the present review will be limited to drugs or drug combinations used in phase II or III trials and to drugs used in adjunctive therapies.

CLINICAL METHODS AND MATERIALS

The information presented here was obtained from the literature and from our unpublished phase II studies. As can be seen from tables 1–3 (*see* end of text), the criteria used to define response to therapy varied widely, and the terms used to evaluate response and survival differed among the several series. We have attempted to evaluate the criteria used in each study, as well as the validity of the conclusions reached. However, the tables reproduce the response and survival data from the original articles.

The primary purpose of a phase II drug trial is to determine the efficacy of a new drug against a given type of tumor, as determined by the resulting increase or decrease in tumor size. In lung tumors, for instance, complete remission is defined as the disappearance of radiographic evidence of tumor for a minimum of 4 weeks, and partial remission is defined as a decrease of ≥ 50% in the size of the lesion for a minimum of 4 weeks, with no appearance of new lesions. With brain tumors, the methods used to determine the percentage of reduction in size for other solid tumors are obviously not applicable. In most reported studies, response was evaluated on the basis of the patient's *clinical improvement.* Occasionally, changes indicated by other combinations of tests, eg, computerized tomographic (CT) scan, radionuclide (RN) scan, and/or angiography, were considered in determining a response. Each of these tests yields results open to variable interpretations; for example, changes in an RN scan may be due to cerebral edema rather than to tumor progression. In our opinion, the most accurate way of rating response is to categorize *as responders* those patients who show improvement in two or more tests (clinical examination, and/or RN scan, and/or CT scan) performed while they are taking stable or diminishing doses of glucocorticoids and to consider patients in whom only one test indicates improvement while others indicate no change to have *stable disease* (8). We have tried to apply these criteria in this review of the chemotherapy literature; in some instances, therefore, our evaluation of the data from a particular report may differ from that of the authors.

Phase III trials, which are designed to compare a drug regimen showing good phase II results against the best current treatment, appear sparsely in the literature, and those that are available have generally reported results in terms of the length of patient survival. Even for these studies, however, we feel that *time to tumor progression* is a better criterion of response.

AGENTS IN CURRENT USE FOR BRAIN TUMOR CHEMOTHERAPY

Antitumor agents currently used in brain tumor chemotherapy are discussed in alphabetical order, followed by separate sections on radiosensitizers and radiopotentiators and on combination-agent chemotherapy.

Adriamycin (Doxorubicin)

At present, no phase II study of doxorubicin (Adriamycin) used alone to treat primary cerebral neoplasms has been published. A major complication of doxorubicin therapy is cardiac toxicity, which can be decreased if the cumulative dose is kept below 500 mg/m² (9). The patients with solid tumors in the study by Benjamin et al (10) had progressive disease of the CNS, and they developed new cerebral metastases while responding systemically. On the basis of this scanty clinical information plus our knowledge of doxorubicin's molecular size and low lipid solubility, its consequent inability to cross the blood-brain barrier, and its myocardial toxicity, it seems unlikely that doxorubicin will prove to be effective as a single agent for primary intracranial neoplasms.

Bleomycin

In a clinical trial in Japan, Kanno et al (11) administered 300 mg of bleomycin in a dosage of 15 mg iv every 4 days to 12 patients with malignant gliomas; clinical improvement was reported in six patients. However, interpretation of these responses is difficult, as many of the patients received radiation therapy concurrently.

In a clinical trial in 1975, an overall response rate of > 50% (61% for gliomas) was reported by Takeuchi (12), who found that bleomycin produced fewer effective responses among highly malignant gliomas and medulloblastomas and was not markedly effective against any of the metastatic tumors. As clinical status was the only criterion used to

evaluate response, it is impossible to distinguish the concurrent effects of surgery, steroid therapy, radiation therapy, and chemotherapy; for instance, five of the glioma patients who showed a marked response received concurrent radiation therapy.

The recommended dosage is 15 mg/m² iv every 4 days for 6 weeks. Fever was a notable side effect in 32% of the patients studied by Agre (13); anorexia, alopecia, and thickening of joints in the hands were also common. In the combined Japanese trials, 1% of the patients died of interstitial pneumonitis; however, this complication was fatal only in older patients receiving doses > 300 mg.

Even with these recent results, phase II trials of bleomycin in brain tumors are still inconclusive.

Dianhydrogalactitol

In 1979, Eagan et al (14) reported the only controlled phase III trial of dianhydrogalactitol (DAG) in the literature, a comparison of whole-brain irradiation alone against irradiation plus chemotherapy with DAG. Forty-two patients who had supratentorial astrocytomas (grade III or IV) were randomized into four treatment groups. All patients had surgery (gross total or subtotal resection in 38 cases, biopsy alone in four) followed in 10–14 days by radiation therapy. Twenty patients received continuous whole-head irradiation (5000 rad delivered in 25 fractions over 5 weeks), and ten of these received, in addition, DAG (25 mg/m²/day iv for 5 days every 5 weeks for the first year and every 10 weeks for the second year). Another 22 patients received split-course irradiation (2500 rad in 10 fractions, with the course repeated following a 3-week rest period), and 11 of these received DAG according to the previously described schedule. While there was no significant difference in duration of survival on the basis of the two radiation schedules (37 weeks with continuous irradiation and 35 weeks with split-course irradiation), the addition of DAG resulted in a prolongation of median survival of 68 weeks, which was statistically significant by the Gehan-Wilcoxon method, even after adjustments were made for sex, age, and tumor grade. The median duration of survival was, however, shorter for older patients in all treatment groups. Patient age was found to affect survival time more than tumor grade. The functional status of the patients, which might prove to be a significant determinant of survival, was not evaluated (eg, by the Karnofsky rating).

The median survival reported for patients receiving only irradiation, which is similar to that for large studies such as the Brain Tumor Study Group (BTSG) cooperative trial (5), was approximately doubled by the adjunct of DAG. Although these results must be evaluated in light of the small number of patients in each treatment group, they are superior to those achieved with any other agent to date. Further evaluation of DAG and similar agents, such as dibromodulcitol, appears to be warranted.

Epipodophyllotoxin (VM-26)

In phase I trials, VM-26 has shown antitumor activity against primary intracranial neoplasms as well as a variety of lymphomas and other solid tumors (15,16). Skylansky et al (17) treated 19 patients who had inoperable or subtotally resected recurrent malignant intracranial neoplasms. Criteria for admission of patients to the study were no prior chemotherapy or either disease progression or excessive cumulative myelosuppression while receiving nitrosoureas. Patients with prior chemotherapy received 100 mg/m² of VM-26, and those with no prior chemotherapy received 130 mg/m²; in both groups, chemotherapy was administered iv in six to eight weekly doses with a 2-week interval between each course. A system for staging CNS disease was used to evaluate these patients: remission was defined as improvement by one disease stage or more maintained for one full course of chemotherapy.

In 13 patients with measurable disease, five objective remissions were seen. The five responders included four patients with malignant primary tumors and one with metastatic anaplastic bronchogenic carcinoma. Improvement occurred gradually, beginning toward the middle of the first course of chemotherapy. The most significant improvement was always seen during the first course. The response lasted for 3–14 months. All seven patients with measurable disease at the start of chemotherapy, including five with malignant gliomas, constituted a subgroup that continued to do well during the initial course of therapy; the median time to progression for this group was 15+ months.

Immediate toxic effects consisted of mild nausea or vomiting. Myelosuppression usually appeared early in the course of therapy and primarily affected hematocrit and leukocyte counts. The greatest absolute decrease in marrow activity occurred during the first course of therapy. No cumulative effect of toxicity was noted, and partial recovery was observed in all cases during the 2-week interval between courses. Only three patients, all of whom had shown severe marrow suppression with nitrosoureas, exhibited clinically significant marrow suppression.

As a single agent, VM-26 produced a 38% clinical remission rate in patients with malignant intra-

cranial tumors, but this was still not as effective as the better nitrosoureas, BCNU and CCNU, or procarbazine. VM-26 has shown antitumor activity in patients who showed disease progression while receiving nitrosoureas. Because these patients appear to tolerate its toxicity well, studies are in progress to determine whether it is effective as an ancillary agent for them. Preliminary data, unfortunately, provide no evidence of any significant antitumor activity.

In 1977, Revira et al (18) used VM-26 to treat 13 children with disseminated neuroblastoma who had become refractory to cyclophosphamide, doxorubicin, and vincristine. At admission to the study, 12 children had had radiation therapy; 11 of these had metastatic bone marrow involvement. A partial response was seen in three patients completing a full course of chemotherapy (cumulative dose, 800 mg/m^2). A complete response (disappearance of all tumor, absence of tumor in the bone marrow, and resumption of normal bone marrow cell production) was not seen in any of the patients.

Neuroblastoma and medulloblastoma are histologically similar, and both appear to be sensitive to various chemotherapeutic drugs. Revira et al indicated that a trial was under way using VM-26 in a sequential combination based on studies of cell kinetics. The results of this may be applicable to patients with medulloblastoma.

Epodyl

Khanna et al (19) reported on a series of 79 patients with histologically verified primary glioma (55, grade I or II; 24, grade III or IV) treated with a dose of 100–200 mg/kg injected into the carotid artery. Prior to administration of triethylene glycol diglycidyl ether (epodyl), some preliminary surgical procedures were performed in all cases: biopsy via burr hole in 46 patients; subtotal resection in 29; and surgery and radiation therapy in four. Clinical improvement of unspecified duration was reported in 46 patients (58%). In 16, there were severe toxic reactions consisting of cerebral edema that resulted in the death of five patients within a few days of administration. In all patients, myelosuppression occurred within 1–2 weeks after infusion and usually resolved within 4 weeks. The authors concluded that patients receiving epodyl survived longer than patients without chemotherapy; however, it is difficult to draw conclusions from this study because (*a*) 55 of the patients treated had low-grade gliomas; (*b*) a response was recorded if any clinical improvement was noted, regardless of its temporal relationship to chemotherapy; and (*c*) in 33 patients, epodyl was used as an adjunct to radiation therapy or surgery.

In a small number of evaluable patients, epodyl has shown minor antitumor activity against both primary and metastatic tumors. This possible effectiveness is, however, overshadowed by its unacceptable toxic effects when administered intraarterially. The Food and Drug Administration Investigational New Drug application has been withdrawn (J. B. Jewel, personal communication, 1970).

5-FU

Edland et al (20) are the only investigators to have published results from a prospective randomized trial (phase III) using 5-fluorouracil (5-FU) against malignant gliomas (grades III and IV). The valid study group was comprised of 30 patients; 16 had radiation therapy (5000 rad in 6–7 weeks) and 14 received radiation therapy and iv 5-FU (10 mg/kg on Days 1–3; 5 mg/kg on Day 4; then 5 mg/kg three times a week for the duration of radiation therapy). Patients who had radiation therapy alone survived an average of 11.7 months from the date of surgery, whereas those receiving 5-FU in addition to irradiation survived 11.5 months. Eleven patients exhibited toxic effects consisting of nausea and vomiting during and shortly after drug administration, as well as mild myelosuppression. In one patient, persistent stomatitis was so severe that it necessitated discontinuation of therapy.

Although 5-FU has never been evaluated in a phase II trial as a single agent against malignant intracranial neoplasms, it would appear from Edland's report to have no significant antitumor activity against brain tumors at the dose schedule used. This could have been anticipated from the data of Hoshino et al (21) on cell kinetics, which demonstrate a low growth fraction (10%–40%) in cells of glioblastoma multiforme. However, recent studies indicate that 5-FU in combination with BCNU may have a significant effect against tumors (*see* Combination-Agent Chemotherapy).

Imidazole Carboxamides (DTIC and BIC)

Kingra et al (22) used DTIC to treat five patients with low-grade gliomas. At the time of progression, the drug was given as an iv bolus for 10 days at a dose of 4.5–6.0 mg/kg of ideal weight; treatment was repeated in 30 days if there was a response (defined as ≥ 50% improvement in neurologic deficit and on a performance scale; improvement indicated by RN scan was not considered). Responses were reported in three of the five patients (one oligodendroglioma, one of the two brain stem gliomas, and

one of the two gliomas) noted to have progressive symptoms after surgery and radiation therapy. Using more stringent criteria, we would classify this as stable disease maintained for 6–18 months.

All patients had some degree of nausea and vomiting, which could not be correlated with the DTIC dose (4.5–6.0 mg/kg). At lower doses (4.5 mg/kg), few patients showed myelosuppression, but patients who received 6.0 mg/kg showed suppression of leukocytes and platelets. Four patients bled from various sites, and one died from myelotoxicity. Hepatotoxicity was also noted at this higher dose range and caused one death.

In a phase II study of childhood leukemia and solid tumors, the Southwest Oncology Group (SWOG) (23) treated ten neuroblastomas and one ependymoma (all recurrent) with iv doses of BIC ranging from 350 to 1100 mg/m²/day for 5 days, repeating courses every 4–6 weeks. They noted a complete response (remission of all tumor size) in one patient and improvement (50% reduction in tumor size or relief of bone pain) in three patients, all with neuroblastoma. The disease of the only patient with primary CNS glioma (ependymoma) continued to progress despite therapy. The duration of response was quite short: all patients died from their tumors within 2 weeks of maximal response. The toxic effects consisted of significant gastrointestinal upset during the course of iv administration and dose-related myelosuppression and hypersensitivity that necessitated discontinuation of therapy.

Finklestein et al (24), of the Childhood Cancer Study Group (CCSG), administered DTIC to 18 children with neuroblastoma and one child with recurrent medulloblastoma. The dose range was 200–450 mg/m²/day iv in 5-day courses repeated every 22 days. The findings were similar to those reported by the SWOG: there were no complete responses, but three (17%) of the 18 patients with neuroblastomas exhibited a partial response that lasted 9–10 weeks. The one patient with recurrent medulloblastoma failed to show any response. Qualitative toxicity similar to that reported by the SWOG was also observed.

In a phase II trial in primary and secondary brain tumors reported by Levin et al (25) in 1975, BIC was administered to 24 patients by rapid iv injection over 30 minutes at a dose of 900 mg/m²/day for 5 days, with retreatment every 6 weeks. Response was defined as unequivocal improvement indicated by both the patient's neurologic status and by RN scan while corticosteroid dosage was unchanged or decreased; probable response was defined as no deterioration evident in the patient's neurologic status or RN scan while steroid dosage remained stable. Four (25%) of the 16 patients receiving BIC as the first form of chemotherapy responded for a median of 5+ months and two (33%) of the six patients who received BIC as a second form of chemotherapy responded for 11.5 and 17+ months. Four (44%) of the nine patients receiving two or more courses of BIC responded, and four (31%) of 13 receiving less than two courses responded. The response rate for all malignant gliomas was four (21%) of 19 patients.

BIC and DTIC have shown only minor antitumor activity in primary cerebral neoplasms. Although these drugs are lipid-soluble, their reported response rates as single agents are disappointing in comparison with the nitrosoureas or procarbazine. In the only reported study in which DTIC has been used in combination with a nitrosourea to treat primary cerebral tumors (26), the results were not significantly better. Further studies with DTIC or BIC are probably not indicated.

Methotrexate

The first trials of methotrexate (MTX) in brain tumor patients incorporated intra-arterial infusions (26–29). Subsequently, Weiss and Raskind (30) instilled MTX topically into the tumors of nine patients (seven glioblastomas, one cerebral hemisphere neuroblastoma, and one meningeal carcinomatosis) by performing a wide internal decompression that left a cavity into which MTX was instilled via a catheter and an Ommaya reservoir. Patients received 0.25 mg/kg in the cavity every 5 days while hospitalized and weekly thereafter (up to 20 injections). Four of the seven evaluable patients were judged to have responded to therapy; two of these were alive at 3 months, one was alive at 5 months, and one was alive at 6 weeks. With this dosage schedule, only myelosuppression was noted.

In a preliminary report, Garfield and Dayan (31) described the use of postoperative intracavitary MTX to treat eight patients with hemispheric malignant gliomas and one patient with metastatic carcinoma. Patients received daily doses increasing from 25 to 200 mg (totaling 300–1250 mg) with citrovorum rescue (CVR) factor (18–36 mg of im leucovorin daily for 4–10 days). Survival of patients treated with MTX was no better (mean, 20.3 weeks) than that expected in patients without chemotherapy. Despite CVR, two patients developed leukopenia.

During studies on the efficacy of MTX in children with acute leukemia, Djerassi et al (32) found substantial concentrations of the drug (0.7–3.25 μg/ml) in the cerebrospinal fluid (CSF) 12–24 hours after its iv administration (15–100 mg/kg in the infusion), so Djerassi et al (33) treated seven children who

had various types of malignant glioma (table 1) in a protocol that specified increasing iv infusions of MTX daily (from 100 mg/kg in 6 hours to 900 mg/kg in 12 hours by Day 77, then every 3 weeks at the highest dose), and then giving CVR (0.2–0.4 mg/kg im every 6 hours for eight doses) when toxicity was observed. Objective neurologic improvement was observed in all except the one patient with medulloblastoma. Five patients survived for 2–13 months; one was still alive at 6 months; and another, who received intra-arterial MTX in addition, survived > 109 months. Despite CVR, six of the seven patients experienced moderate to severe toxicity, including stomatitis, skin rash, and myelosuppression; but the toxicity was not associated with mortality.

In autopsies of patients with metastatic osteogenic sarcoma who had received high doses of MTX (200–500 mg/kg) with CVR, Rosen et al (34) found normal histopathology of liver and brain. Analysis of these tissues revealed accumulation of MTX as well as CVR, occasionally in larger amounts than MTX (M. Rosen, unpublished data). Because this suggested that CVR may protect the normal brain from the leukoencephalopathy associated with irradiation and systemic MTX, these investigators designed a protocol whereby recurrent childhood brain tumors were initially evaluated for response to high-dose MTX (HDMTX) (500 mg/kg) with CVR (10 mg, orally every 6 hours for 11 doses); HDMTX was then given on a biweekly schedule with vincristine (115 mg/m² iv every 2 weeks) and a nitrosourea, either BCNU (90 mg/m²/day iv for 2 days) or methyl-CCNU (120 mg/m² orally every 6 weeks).

Following irradiation (4000–6000 rad), 11 children with recurrent tumors (seven medulloblastomas, four pontine gliomas) were treated with this schedule. Five of the seven patients with medulloblastomas showed tumor regression indicated by serial CT scans, and in two of these patients, all evidence of enhancing tumor disappeared. The median duration of response in the five responders was 9 months (range, 6–34+ months). Of the four patients with brain stem glioma, only two exhibited objective evidence of response to MTX. One response, which was documented by serial pneumoencephalography, lasted 6 months, and one patient showed dramatic improvement in neurologic signs and symptoms for 18 months.

Drug levels in serum and CSF following MTX therapy were evaluated in four patients. Concentrations of MTX in CSF peaked at 1×10^{-5} M at 12 hours after the initial 4-hour HDMTX infusion, with detectable levels for up to 50 hours after administration.

Systemic toxicity, which was seen in all patients, was limited to mucositis and myelosuppression. Nausea and vomiting attributable to the nitrosourea therapy were also observed. One patient developed leukoencephalopathy 6 months after the start of MTX therapy and 6.5 months after radiation therapy (6000 rad to midcerebral fossa). Whether this is attributable to HDMTX or to irradiation is unclear; however, Griffin et al (35) recently showed in mice that photon irradiation (2000 rad) allowed detectable levels of MTX across the blood-brain barrier. This suggests that the combination of irradiation and HDMTX may be responsible for the unexpected neurotoxicity.

On the basis of the responses in children reported by Djerassi and Rosen, Shapiro (36) treated six adults who had high-grade malignant gliomas, five of which showed evidence of progression. MTX was administered at a dose of 500 mg/m², preceded 1–4 hours by vincristine (1 mg/m² iv) and followed by CVR 24 hours after the start of therapy. Patients received from two to seven treatments at maximum doses ranging from 27 to 522 mg/kg/treatment. All but one patient developed mild mental clouding that progressed to severe stupor and coma 2–4 days following MTX treatment. As a result of these severe toxic responses, the study was discontinued. More importantly, five of the six patients had disease progression despite MTX treatment; they were subsequently treated with other unspecified chemotherapeutic regimens and reported to be stable or responding.

In summary, the severe toxicity associated with HDMTX makes it of questionable value for malignant glioma in adults. Although it has shown promise in the treatment of brain tumors in children, optimal schedules and combinations with other drugs and/or radiation therapy are yet to be worked out.

Mithramycin

Clinical studies prior to 1970 (37–39) suggested that mithramycin was active against recurrent or inoperable malignant intracerebral neoplasms. Radioactive mithramycin given iv appeared in the CSF, with a maximum concentration (blood concentration = CSF concentration) at 4–6 hours (38).

In an attempt to lessen gastrointestinal toxicity seen with iv administration, Mealey et al (40) administered mithramycin intra-arterially in lower doses to eight glioblastoma patients. They showed no clinical improvement and survived for 3 days to 4 months. Although the hemorrhagic manifestations of toxicity were obviated, four patients developed hemiplegia secondary to carotid artery infusion.

Laboratory studies indicated that mithramycin

produced a modest inhibition of growth (30%–40%) in Zimmerman ependymoblastomas implanted subcutaneously in mice (41), as well as producing unique toxic changes in the cell nuclei in cultures of a variety of neural tumors, including many malignant gliomas (42).

On the basis of this clinical and laboratory data, the BTSG designed a phase III study, conducted by Walker et al (43) in 1976, in which 96 patients who had anaplastic gliomas were treated with mithramycin. They showed no statistically significant difference in survival (21 weeks vs 26 weeks for controls) as a result of treatment with mithramycin alone. Significant improvement was defined as an increase of ≥ 75% over the control group in median survival time. Patients were randomized into two groups 6 weeks after surgery; one group received radiation therapy but the groups were comparable in all other parameters, including postoperative radiation therapy (55% in each group). For patients with postoperative radiation therapy, the median survival time was 36 weeks, compared with 15 weeks for patients who did not receive postoperative radiation therapy ($P \leq 0.05$). Adults younger than 40 survived longer than those older than 60. Survival correlated with duration of symptoms, with median survival times of 9, 29, and 45 weeks, respectively, for those having had symptoms for < 26 weeks, for 26–52 weeks, and for > 52 weeks. Mithramycin produced toxic complications consisting of myelosuppression (60% of patients), gastrointestinal hemorrhage (58%), and changes in liver function tests (31%); three patients (3%) had intratumor hemorrhage.

Although mithramycin has been measured in the CSF, its molecular weight (> 1000 daltons), poor lipid solubility, and high plasma protein affinity all contribute to make the blood-brain barrier impermeable to it. More importantly, it has not shown significant antitumor activity in well-controlled phase II and III studies.

Nitrogen Mustard (Mechlorethamine)

Mechlorethamine is one of the oldest chemotherapeutic agents in use (44). Although it was the first drug tried in brain tumor patients (45), only anecdotal information is available on its effectiveness. All reported studies have been uncontrolled and have used mechlorethamine infused or perfused intra-arterially as an adjunct to surgery and/or irradiation.

In 1969, Owens (46) treated 15 glioblastomas and six metastatic tumors with intra-arterial mechlorethamine infusions, diminishing the toxicity by the addition of iv infusions of Na thiosulfate (250 mg/mg of HN_2). None of these patients showed evidence of clinical response or prolonged survival. Despite the fact that Na thiosulfate prevented hematologic complications, four patients died from toxic cerebral edema. All patients developed transitory ipsilateral ophthalmoplegia, and a significant number had occlusions of the internal carotid artery. These results were quite discouraging at the time, although in retrospect it can be appreciated that this work provided the impetus for further investigation of other drugs and routes of administration for the treatment of patients who have brain tumors.

Nitrosoureas

In 1962, Rall and Zubrod (47) characterized the ideal compound for crossing the blood-brain barrier by diffusion: high lipid solubility, low ionization at physiologic pH, and low plasma protein affinity. These characteristics are possessed by all three of the nitrosourea derivatives—BCNU, CCNU, and methyl-CCNU—that have been found active against intracerebral tumors. All produce dose-limiting myelosuppression in man. Platelets and leukocytes are the blood products most severely affected. Myelosuppression is usually delayed; it begins to occur at 35 weeks and lasts approximately 21 days (48). Cumulative myelosuppression occurs after 35 courses (49). Clinically, acute nausea and vomiting occur in 50% of patients 3–4 hours after either iv or oral administration. Other less frequently observed effects include stomatitis, alopecia, anemia, anorexia, hepatotoxicity, and decreased pulmonary reserve. Although excretion of nitrosourea metabolites is primarily renal, nephrotoxicity has not been noted in man (50).

Pharmacokinetic studies in man and in animals indicate rapid degradation of the parent compound (51). It has been possible to isolate the intact drug from both blood and CSF samples but not from urine (52). Due to their high lipid solubility, all three nitrosoureas cross the blood-brain barrier rapidly. CSF levels usually reach 15%–30% of simultaneous blood levels.

Whether both the antitumor activity and the myelosuppression are produced by the same metabolite is not known, although there are recent indications from studies in animal models that they are not.

BCNU

BCNU has been evaluated in numerous phase II and III trials.

In 1970, Broder and Carter (53) of the Central Oncology Group noted two partial clinical responses

in four patients with recurrent malignant gliomas treated with an iv 5-day course of BCNU. Toxicity was limited to myelosuppression.

In an uncontrolled phase I–II trial, Walker and Hurwitz (54) treated 27 patients who had primary or secondary malignant brain tumors. Three different regimens were evaluated. Intensive intermittent therapy (100–125 mg/m² for 3 days) produced dramatic clinical improvement in ten (62%) of 16 glioma patients for 3–18 months; five (50%) of the responders had malignant gliomas. Although clinical response was dramatic, no positive correlation could be made by concomitant RN scanning. In 22 patients with primary brain tumors, an overall response rate of 45% (ten patients) was achieved. The most profound improvement was noted at 34 weeks after the first treatment. As expected, toxicity was most severe in the patients receiving intermittent intensive therapy. Platelets were severely reduced, necessitating platelet transfusion in 50% of the patients receiving BCNU.

In a nonrandomized phase II study of 36 recurrent primary brain tumors, Wilson et al (55) noted an overall response rate of 47%. A response was recorded if clinical improvement began > 3 weeks after the start of steroid therapy at a constant or decreasing dose or if improvement seemingly due to this therapy persisted after discontinuation of steroids. Nine of the responding patients did not receive any steroids. Responses were noted in seven (39%) of 18 patients with glioblastoma multiforme and in four (66%) of six with astrocytomas. Intensive intermittent therapy, using BCNU at 60–100 mg/m² iv for 3 days every 6–8 weeks, produced myelosuppression of all bone marrow elements. Although 19 of 34 patients required blood transfusions sometime during therapy, no serious complications were reported. In most responders, clinical improvement preceded a decrease in the size of the permeability defect evidenced by serial RN scans.

In a nonrandomized phase III trial, Armentrout et al (56) treated 27 patients who had a pathologically diagnosed malignant glioma. Eleven patients who had surgery followed by irradiation (6000 rad in 7–8 weeks) survived a median of 30 weeks; 16 patients who received BCNU in addition to surgery and irradiation survived for a median of 43 weeks. At 1 year, only 14% of patients who had not received chemotherapy survived, but in the treated group the survival rate was 55%.

In a controlled, randomized, prospective phase III study, Walker et al (5) of the BTSG treated 303 patients who had anaplastic gliomas. The valid study group comprised 222 patients, who were randomized into four subgroups. Glucocorticoids were not allowed. The individuals who received the best conventional care (ie, surgery but not chemotherapy or irradiation) survived for a median of 14 weeks; patients who received at least one dose of BCNU (80 mg/m² for 3 days every 6—8 weeks) survived for a median of 18.5 weeks; those who had postoperative irradiation survived for a median of 35 weeks; and those who received BCNU in conjunction with irradiation at the same dosage survived for a median of 34.5 weeks. If only those patients considered adequately treated are taken into account, then the median survival times were 17.0 weeks for surgery alone, 25.0 weeks for BCNU, 37.5 weeks for irradiation, and 40.5 weeks for irradiation and BCNU together; but the difference in survival with radiation therapy alone and that attained with BCNU in addition to radiation therapy was not statistically significant. By the Wilcoxon signed-ranks test, all other forms of treatment were better than surgery alone ($P < 0.003$). However, at 18 months, 19% of patients receiving BCNU and irradiation were alive, compared with 4% of patients receiving only BCNU. This difference is statistically significant to the 0.01 level. Factors found to be important determinants of the prognosis were: the patient's initial performance status (Karnofsky rating), the patient's age, the extent of surgery, and the nature of the symptom complex (eg, seizures or cranial nerve involvement).

CCNU

In a brief letter in 1971, Hansen and Muggia (57) reported the use of CCNU at a dose of 130 mg/m² to treat six patients who had recurrent malignant gliomas (grade III and IV astrocytomas). Response (functional improvement for at least 2 months) was noted in five patients (83%), three of whom were bedridden at the start of treatment but ambulatory when discharged from the hospital. Clinical improvement was noted for 3–12 months (average: 5.5 months) after the start of therapy.

In 1972, Fewer et al (49) reported the results of a study carried out at the School of Medicine, University of California, San Francisco (UCSF), where CCNU was used to treat recurrent malignant primary or metastatic tumors. The valid study group consisted of 29 patients treated with 120–130 mg/m² every 6–8 weeks. None of the eight patients who had previously received BCNU chemotherapy showed a response, but seven (33%) of the 21 patients who had not previously received BCNU responded. Five of the seven responders were patients with glioblastoma multiforme. Myelosuppression was the dose-limiting manifestation of toxicity, but cumulative toxicity was not significant

with this dosage schedule. Toxic effects were more profound in patients who had previously had BCNU therapy. A very high correlation was noted between the clinical course and the results of RN scan, suggesting that serial scans could be used as a reliable measurement for evaluating response to therapy.

Although CCNU has the advantage of being effective if administered orally, it is less effective than BCNU when used as a single agent for recurrent tumors. Rosenblum et al (58) conducted a phase II trial in which 26 patients with recurrent malignant primary or metastatic tumors were treated with oral CCNU at a dose of 130 mg/m². Doses were adjusted for myelosuppression and repeated every 6–8 weeks. The valid study group (patients receiving two or more courses of CCNU) consisted of 24 patients. A response rate of 43% was found for all patients with primary tumors, regardless of prior irradiation; 37% of the treated patients experienced significant clinical remission, the median lasting 5 months. Responders survived for a median of 15+ months. Once progression of disease had been observed, however, continued CCNU therapy failed to elicit improvement. Nausea and vomiting were experienced by 70% of the patients 4–6 hours after administration; this was lessened but not eliminated by pretreatment with antiemetics. Delayed myelotoxicity caused marrow levels to reach a nadir at 3 weeks posttreatment, usually recovering to pretreatment levels by 5 weeks. After four or five courses of CCNU, there was cumulative toxicity similar to that caused by BCNU. Platelets were more severely affected than leukocytes. One patient died of sepsis secondary to myelosuppression, and three deaths were considered to be drug-related. As with BCNU, there was no evidence of significant hepatic or renal toxicity.

In a large, combined phase II study, Hoogstraten et al (59) administered CCNU to 141 patients with a variety of advanced malignancies. Initially, the dose was 130 mg/m² adjusted for myelosuppression. Courses were repeated every 6 weeks. Five (36%) of 14 patients with malignant brain tumors improved clinically. Responses lasted only 4–19 weeks in patients with brain tumors. The majority of responses were noted 2–4 weeks after the first course of chemotherapy. In contrast to experience at UCSF, these investigators found no correlation between prior treatment with a classic alkylating agent and response to CCNU.

Weir et al (60) conducted a three-arm phase III study of CCNU involving 41 consecutive patients with supratentorial brain tumors (38 malignant gliomas). Within 2 weeks of surgery, patients were randomly allocated to one of three groups. One received irradiation (4000–4500 rad for 5 weeks), one CCNU (130 mg/m² every 6 weeks), and one a combination of irradiation and CCNU. When progression of disease was observed in patients receiving CCNU or irradiation only, they were crossed-over to the other form of therapy. A response was recorded if a patient showed neurologic improvement on the basis of RN scan, EEG, or both, without showing disease progression according to the other parameters or if clinical grading indicated maintenance of improvement for at least 4 weeks. For patients who had radiation or CCNU therapy, the median times to crossover were 23 and 14 weeks, respectively, whereas patients treated with combined therapy exhibited a median time to progression of 31 weeks. Median survival was recorded as 27 weeks for patients treated with irradiation, 37 weeks for those who received CCNU, and 36 weeks for those who received the therapy combining irradiation and CCNU. The difference among the three treatment groups was not statistically significant, either in duration of survival or in time to progression. Although the concurrent use of irradiation and CCNU was not statistically better than either treatment alone, patients who had the sequential combination of the two therapies as a result of crossover exhibited a 25% increase in mean survival time. Of the seven patients who were crossed-over to CCNU following recurrence of symptoms after irradiation, only one showed improvement; however, five of the ten patients who had irradiation after a relapse during CCNU treatment showed significant secondary improvement. CCNU toxicity was generally mild, necessitating dosage reductions in only six patients.

In 1976, Keagan et al (61) reported the results of a phase III trial at the Mayo Clinic involving 63 patients with astrocytomas proven malignant by biopsy. Within 2 weeks of surgery, patients were randomly assigned to one of three treatment groups: 22 patients received only irradiation (5000 rad for 5–6 weeks) and survived a median of 11.5 months; 22 patients received only CCNU and survived a median of 6.6 months; 19 patients were treated concurrently with irradiation and chemotherapy and survived a median of 12.0 months. Radiation therapy alone or in combination with CCNU prolonged survival to a statistically significant extent ($P = 0.02$) beyond that achieved with chemotherapy alone. Moreover, the addition of CCNU to irradiation did not result in a statistically significant prolongation of survival.

In 1978, the European Oncology Radiation Trial Committee (EORTC) (62) completed two large, randomized studies of patients who had grade III or IV astrocytomas. The first trial (No. 26741) evaluated the effectiveness of CCNU as an adjunct to

irradiation in 111 patients, 81 of whom were evaluable. All patients had histologically proven malignant gliomas. All had surgery and subsequently received irradiation (5000–6000 rad; the radiation schedule was not described). Study patients were restricted to those with minimal postoperative neurologic deficit and not requiring steroids. They were randomized into two groups: one received CCNU immediately following radiotherapy, and the other was only followed from the time of radiotherapy until recurrence and then received CCNU (130 mg/m² orally every 6 weeks). The mean disease-free interval for patients treated with CCNU immediately following radiotherapy (34.5 weeks) was no different from that of the control subjects (31.0 weeks). Patients receiving CCNU at relapse survived significantly longer (a median of 22.5 weeks from the time of recurrence, or 62.5 weeks from the time of surgery) than those receiving CCNU immediately after surgery (11 weeks from recurrence, or 43 weeks from surgery). Younger patients (< 50 years) and patients with tumors other than glioblastoma multiforme tended to survive longer.

The second trial evaluated the effectiveness of CCNU at recurrence in 23 other patients who had greater neurologic dysfunction requiring steroids. All had undergone similar surgery and had similar histologies. They received similar radiotherapy, but ten received CCNU every 6 weeks, while 13 received no chemotherapy. The group that received CCNU had a significantly longer median survival time, 31 weeks compared with 21.5 weeks. Mean survival was generally shorter in patients with more severe postoperative neurologic deficits, patients > 50 years old, and those requiring continued steroids.

The aggregate results of these two trials suggest that CCNU is not effective as an adjunct to surgery and irradiation but does significantly prolong survival when administered at the time of recurrence.

In summary, CCNU as a single agent has not been found to be as effective as BCNU. Toxic side effects are similar in course and severity to those of the other nitrosoureas. The major advantage of using CCNU is the convenience of being able to administer it orally.

Methyl-CCNU

In a phase I–II trial, Young et al (63) used methyl-CCNU (MeCCNU) to treat 17 patients for advanced cerebral lesions (including 11 malignant gliomas and four malignant astrocytomas). Patients were given MeCCNU orally every 6 weeks in doses of 40–290 mg/m². Although no complete regressions of tumor were noted, partial responses were seen in patients receiving doses > 170 mg/m². Responses for a duration of 5+ months were recorded in two (13%) of the 15 patients with malignant primary tumors. Nausea and vomiting occurred routinely when doses exceeded 170 mg/m². Delayed marrow suppression was similar in severity and course to that caused by the other nitrosoureas. Significant hepatic or renal toxicity was not observed. After oral administration of radiolabeled MeCCNU, CSF levels of the drug at any given time were 10%–20% of those in plasma. Although the response rate was low, this trial suggested that doses of 170–220 mg/m² can usually be tolerated without excessive toxicity.

In 1975, Tranum et al (SWOG) (64) treated 29 recurrent malignant gliomas (grades III and IV) with MeCCNU in oral doses of 200 mg/m² every 6 weeks. At least two courses of therapy were considered necessary for an adequate trial. Response to therapy was determined on the basis of changes indicated by RN scan and/or neurologic examination that were sustained after discontinuation of corticosteroids. By this definition, eight (28%) of 29 patients showed a response (one complete and seven partial). The point at which there was a 50% chance of remaining in remission was calculated to be 44 weeks. In contrast to other studies, this trial found that patients who had had nitrosourea therapy responded to MeCCNU. Myelosuppression was dose-related and cumulative and was more severe in patients who had had radiotherapy and/or prior chemotherapy.

In 1978, Walker et al (5) reported the results of the phase III protocol of the BTSG that involved the use of MeCCNU in two of four treatment groups. Although this study is no longer accruing patients, it is still continuing, and the results are only preliminary. The 349 patients enrolled in the study were randomly assigned to receive either (*a*) MeCCNU (220 mg/m² orally every 6–8 weeks), (*b*) irradiation (6000 rad whole head for 6 weeks), (*c*) a combination of MeCCNU and irradiation using the same schedules, or (*d*) a combination of BCNU and irradiation that had produced the best results in their previous study (BCNU, 80 mg/m²/day iv for 3 days every 6–8 weeks and irradiation, 6000 rad in 6–8 weeks). Preliminary data on 253 adequately treated patients indicate that the use of MeCCNU alone resulted in a median survival time of 36 weeks, and MeCCNU in combination with irradiation resulted in a median survival time of 34.6 weeks, while BCNU in combination with radiotherapy proved, as in earlier studies, to be more effective than any of the other forms of therapy, producing a median survival time of 50.3 weeks.

This is better than the results reported for a previous study, but no explanation was given for the improvement.

In 1976, Brisman et al (65) reported on the data from Columbia College of Physicians and Surgeons, which had been incorporated in the findings of the BTSG. From this report, it is impossible to deduce the efficacy of any single chemotherapeutic agent because a different nitrosourea was tested each year: in 1970, BCNU (100 mg/m² iv for 2 days every 6–8 weeks); in 1971, CCNU (100 mg/m² orally once every 6–8 weeks); and in 1972, MeCCNU (150 mg/m² orally once every 6–8 weeks). The study group consisted of 62 patients with histologically verified glioblastomas. Subgroup A consisted of all 62 patients who had craniotomy and within 4 weeks were randomly assigned to receive either irradiation alone or irradiation in combination with a nitrosourea, as described previously. Fifty percent of the 23 patients who received chemotherapy lived for > 12 months. Fifty-one percent of 39 patients who did not receive chemotherapy lived for > 4.2 months. However, five patients (13%) of the group receiving chemotherapy lived for > 24 months. Although treatment with chemotherapy thus appears to give better results, the sample is somewhat biased because the group not receiving chemotherapy included a greater number of older patients with severe postoperative deficits.

Subgroup B consisted of 40 patients with glioblastoma who had surgery and completed a full course of irradiation (6000 rad). Fifty-three percent of the 19 patients who received chemotherapy were alive at 12 months (one survived 24 months). Fifty-one percent of the 21 patients who did not receive chemotherapy survived for ≤ 9 months (five survived > 24 months). However, the differences in duration of survival were not statistically significant ($P = 0.05$).

Subgroup C consisted of 33 patients who, after surgery, were randomly assigned to receive either chemotherapy and irradiation (17 patients) or irradiation alone (16 patients). The valid study group consisted of 24 patients, 12 receiving chemotherapy and 12 receiving irradiation. The point of 50% survival was 6.1 months in the group receiving chemotherapy and radiation therapy and 6.3 months in the group receiving radiation therapy only.

Analysis of the Columbia data revealed that a shorter survival time was associated ($P < 0.05$) with the presence of a severe neurologic deficit 1–2 weeks after surgery, an age > 64 years, or the presence of symptoms for < 12 months prior to surgery. Toxic effects in patients receiving chemotherapy consisted of myelosuppression and mild nausea and vomiting. Hepatitis occurred in one patient. There were no drug-related deaths.

Brisman concluded that there was no increase in survival as a result of chemotherapy, but this conclusion is questionable in view of the fact that the study used several different nitrosoureas, some of which are known to have only minor effectiveness, particularly MeCCNU.

All of the nitrosoureas have shown effectiveness in treating primary cerebral neoplasms, but BCNU is more effective as a single agent than CCNU or MeCCNU. In well-controlled phase III studies, patients treated with surgery and postoperative radiotherapy followed by a 3-day schedule of BCNU every 6–8 weeks have had the best quality of life, as well as the longest median survival time.

Procarbazine

Kumar et al (66) used procarbazine (PCB) in a phase II protocol to treat recurrent primary tumors, intracerebral metastatic tumors, and deep-seated (thalamic or brain stem) mass lesions presumed to be malignant on the basis of neurodiagnostic tests. PCB was given orally at a dosage of 150 mg/m²/day in 28-day cycles, with a 28-day rest period between cycles. The dosage of subsequent courses was adjusted for myelosuppression.

Although 43 patients were treated, the valid study group consisted of 29 patients. Those who showed unequivocal neurologic improvement indicated by both clinical status and RN scans, with a constant or decreased steroid dosage, were considered responders (ie, stable disease); patients who improved in one of the two parameters were considered probable responders. By these criteria, of the 29 patients, 12 (41%) were classified as having stable disease and two (7%) were considered to be probable responders. Of all tumors treated, medulloblastomas showed the best response rate (75%; three of four). The duration of response ranged from 2 to 19 months, with a median of 7 months.

Toxic effects consisted of nausea and vomiting, usually in the first few days of each course of therapy; 75% of the patients exhibited myelosuppression, especially of leukocytes and platelets. Severe bone marrow depression required reverse isolation or other supportive measures in eight patients (19%). Allergic skin reactions were also noted in a small percentage of patients.

PCB used as a single agent has shown significant activity against primary malignant brain tumors. Patients who have received prior nitrosourea therapy have shown some cross-resistance to PCB therapy. When used in combination with CCNU and vincristine, PCB has shown marked antitumor ac-

tivity in patients with malignant glioma and medulloblastoma (*see* Combination-Agent Chemotherapy).

ThioTEPA

The literature provides only anecdotal information concerning the activity of thioTEPA against primary brain tumors (67–69). However, thioTEPA has shown activity against meningeal leukemia when administered intrathecally (70,71). The efficacy of intrathecal thioTEPA against disseminated primary tumors has not been evaluated.

We (72) recently investigated the effectiveness of iv thioTEPA against malignant gliomas in a phase II trial (15–60 mg/m^2 every 3 weeks); and, except for significant myelosuppression, there were no responses in 12 patients. We therefore feel that thioTEPA is ineffective as a single agent for primary brain tumors. Its usefulness in combination with other agents is yet to be established.

Vincristine

In 1969, Owens (46) summarized his extensive experience with intra-arterial vincristine (VCR) used as an adjunct to surgery in patients with malignant gliomas. Patients were treated with intermittent infusions of 1–2 mg/day, to a total dose of 10–15 mg. Analysis of the first 22 patients suggested that those treated ≥ 3 weeks after surgery had a shorter survival time. Postoperative brain scans indicated that the maximum leakage in the blood-brain barrier persisted for 1–7 days after surgery, which encouraged Owens to administer therapy within 5–7 days after surgery. An unusual aspect of the treatment was that all patients had a wide tumor decompression, and the bone flap was discarded. At the time of this report, 30 patients had been treated; 26 had died and their average survival time after the appearance of symptoms was 14 months. Four were still alive, having survived an average of 35 months since the appearance of symptoms. Dose-limiting toxic symptoms consisted of nausea, ileus, conjunctival edema, and erythema of the forehead. Peripheral neuropathy did not occur, even when the doses exceeded total iv doses that usually produce symptoms.

Smart et al (73) studied a series of 176 patients with various malignant neoplasms including 21 unspecified CNS tumors. The first patients were treated with VCR at 0.04–0.08 mg/kg/week iv, but neurotoxicity required a reduction to 0.015–0.03 mg/kg/week. If patients receiving concomitant irradiation are eliminated, responses (50%–100% improvement in grades for neurologic function) were seen in four (25%) of 16, with an additional four showing a lesser response (25%–50% improvement in neurologic function). The four responses were noted in patients who had CNS tumors (one medulloblastoma, one cervical cord astrocytoma, and two malignant supratentorial gliomas). The lack of information on either the timing of operations in relation to chemotherapy or the use of adjunctive steroids makes it difficult to evaluate these results. Neurotoxicity occurred within 1–3 weeks, even in patients receiving the lowest dosage. Psychic depression was attributed to VCR at high doses.

In 1969, Braham et al (74) treated four patients who had symptons of recurrent glioma or whose condition continued to deteriorate after surgery and irradiation. Patients received weekly iv doses, which were gradually increased from 0.05 mg/kg to 0.1 mg/kg over a course of 3 months. When applicable, courses were repeated after a 4-week rest period. All four patients were described as having a good or complete remission of symptoms and signs for 3–18 months. Side effects (gastrointestinal symptoms and transient leukopenia) were described as mild and never severe enough to warrant cessation of therapy.

In 1973, Afra (75) reported on nine patients with recurrent malignant gliomas (astrocytoma, glioblastoma, or medulloblastoma) treated in a phase II trial using VCR given in weekly iv infusions of 2.0–3.0 mg (0.02–0.05 mg/kg) with total doses of 11–22 mg. Seven of the nine patients survived in a reportedly satisfactory neurologic condition for 3–14 months (mean, 6.3 months) after the start of therapy. Toxicity was similar in quality, course, and severity to that in previously reported series.

The most recent reported study is that of Rosenstock et al (76), in which 17 children with clinically recurrent primary tumors were treated with iv VCR at a dose of 1.5 mg/m^2 (2 mg maximum dose) weekly for 12 weeks and thereafter on alternate weeks. Eight of the 16 patients who received an adequate trial of chemotherapy (at least 4 weeks) showed significant objective responses (stabilization or improvement of neurologic status) lasting for a median of > 29 months. Of the eight patients, two had pontine gliomas, one had an optic glioma, two had malignant gliomas, and three had medulloblastomas. Actually, not all of these patients should be classified as true responders (ie, patients with stable disease), nor can the duration of response be meaningfully evaluated for tumors so variable in their natural histories as optic glioma, pontine glioma, and medulloblastoma. Toxic effects consisted of gastrointestinal symptoms (eg, constipation) and mild myelosuppression; two patients required blood transfusions to correct hemoglobin levels between 9 and 10 g. It is unclear whether the development of seizures in three patients during

their first few treatments with VCR was related to the chemotherapy.

Although a carefully controlled trial has never been conducted, VCR as a single agent has been reported to show mild to moderate antitumor activity. It is useful in combination-drug schedules because it does not cause severe myelosuppression.

RADIOSENSITIZERS AND RADIOPOTENTIATORS

Bromouridine

Extensive studies of 5-bromo-2′-deoxyuridine (a thymidine analog), commonly called bromouridine (BUdR), have been conducted by Sano et al (results presented to the American Association of Neurological Surgeons, Boston, Apr 17, 1972). BUdR appears to incorporate itself into the DNA of dividing cells in place of thymidine, purportedly increasing the radiosensitivity of these cells by a factor of 2–3. BUdR has been found to have almost no cytocidal or antimetabolic effects on unirradiated cells. In vitro sudies indicate that the rate of incorporation of BUdR into DNA should be much higher in tumors than in surrounding normal tissue, with a resulting enhancement of the cytotoxic effect of radiation on the tumor cells. Not all tumors take up BUdR because some tumor cells are able to biosynthesize the thymidine they need for DNA replication. If, however, this biosynthetic pathway is blocked by a small amount of an antimetabolite, such as MTX or 5-FU, the replicating cells will be forced to incorporate the thymidine analog, BUdR.

Since 1965, Sano et al have used BUdR in the following protocol: 600–1000 mg of BUdR is administered along with an antimetabolite (0.5–3.0 mg/day of MTX or 25 mg/day of 5-FU) by infusion into the carotid artery (BUdR cannot be administered iv because > 90% would be dehalogenated by the liver in < 1 hour). Irradiation is begun 7–14 days after the first infusion, in a daily dose of 150–200 rad until a total of 5000–6000 rad has been administered. BUdR is infused for 4–7 weeks, with total doses averaging 25,000 mg (maximum, 45,000 mg). As of 1972, 135 patients with various types of tumors had received a full course of BUdR therapy, but response rates or criteria for response have not been reported. In patients with moderately malignant gliomas (grade I or II), the 1-, 2-, and 3-year survival rates were 90%, 82%, and 64%, respectively, whereas patients with highly malignant gliomas (grade II or IV) had 1-, 2-, and 3-year survival rates of 59%, 36%, and 15%, respectively. The incidence of recurrence increased markedly in patients followed for at least 2 years.

The serum bromine levels of patients receiving BUdR should be checked frequently to avoid development of the toxicity of acute bromism. Approximately 2–3 months after begining therapy, patients experienced onychomadesis of the fingers and, occasionally, the toes; this was usually transient. Severe alopecia and radiation dermatitis of the scalp were noted on the side of infusion in all patients. Vascular complications, which are predictable with any arterial infusion, occurred in 13 patients (10%). The majority of these were asymptomatic carotid occlusions; only one patient sustained a significant neurologic deficit (hemiparesis, probably secondary to air embolism).

Infusion of BUdR is a complicated procedure requiring hospitalization for 5–7 weeks and meticulous care to prevent complications of the infusion system. Although the data for moderately malignant gliomas look impressive, these patients actually had low-grade gliomas (grades I or II) (T. Hoshino, personal communication, 1978). Patients with tumors extending across the midline were not treated with BUdR, so highly malignant gliomas were likely to be excluded, and this study is biased by patient-selection criteria.

Hydroxyurea

In 1970, Lerner et al (77) used hydroxyurea (HU) and irradiation to treat 15 patients who had histologically proven malignant gliomas. After operation, patients were given 80 mg/kg orally every 3 days; 7 days after the start of HU, a 5-week course of irradiation was begun (total dose, 4000 rad). Patients continued to receive HU indefinitely at the same dosage unless the wbc count decreased below 2500/mm^3. Patients completing irradiation and HU therapy had a median survival time of 13 months. The only significant side effect was mild myelosuppression, which quickly resolved after discontinuation of therapy. It is difficult to evaluate these results because the patients received significantly lower doses of radiation than are usually used now.

Irwin et al (78) used HU and radiation therapy to treat 27 patients who had malignant gliomas in a randomized phase III study. Because extensive tumor necrosis had been noted in the first group at autopsy, 29 patients were studied using a modified schedule that included a second operation for elective removal of necrotic tumor on completion of therapy. Patients received either a placebo or HU at a dose of 10 mg/kg every 6 hours, starting 1 week prior to radiation therapy (4800 rad, whole-brain and tumor portal) and continuing throughout its course. The 14 patients receiving the placebo survived for a median of 8 months, compared with 12 months for the 15 patients receiving HU. There is no mention of toxic effects in the report. Although

the median survival time of the treated group was significantly longer, the dose of radiation administered was low. There are at present two phase III studies using HU in combination with radiation therapy (6000 rad, whole-brain) followed by BCNU or PCB, CCNU, and VCR in combination (PCV). These should provide valuable information on the efficacy of HU.

Metronidazole

Metronidazole (Flagyl) has shown capacity in vitro to radiosensitize hypoxic mammalian cells, as well as cells from various animal tumor models (79–81). Urtasun et al (82) reported a phase III trial using metronidazole in conjunction with irradiation (3000 rad in 18 days) in patients with supratentorial glioblastomas (grade IV). After surgery, 15 patients randomly assigned to receive irradiation alone had a median survival time of 15 weeks, while 15 patients given metronidazole (6 g/m^2 orally 4 hours prior to radiation therapy three times a week every third week) in addition to irradiation had a median survival time of 26 weeks. The superiority of survival in patients receiving metronidazole was significant ($P = 0.02$). Although the failure rates in the two groups were equal, patients receiving metronidazole showed a delay of 4.5 months, on the average, before their condition worsened. Patients receiving only irradiation exhibited a poor median survival time, compared with patients in previous studies (*see* Combination-Agent Chemotherapy), while those receiving metronidazole approached the median survival time usually associated with irradiation alone (29–33 weeks). This is probably attributable to the less than ideal fraction of irradiation. Patients who received metronidazole immediately experienced toxic reactions, manifested as nausea and vomiting. No myelosuppression was noted, and peripheral neuropathy was not mentioned in the report. There is recent evidence that total doses > 100 g may cause permanent damage to the peripheral nervous system.

Misonidazole

In 1979, Carabell et al (83) reported preliminary results of a phase II trial of irradiation and misonidazole used to treat 41 patients with grade III or IV astrocytomas. Misonidazole (2.5 g/m^2) was administered orally 4 hours prior to each 400-rad fraction of whole-brain radiotherapy (administered over 6 weeks: 400 rad on Day 1 of each week and 150 rad/day during the remainder of the week), a total of 15 g/m^2 of misonidazole and 5100 rad, which was followed in 5 days with a 900-rad boost delivered either to the tumor or to the whole brain (for a total of 6000 rad in 7 weeks). Of the 41 patients, 31 were alive after followup intervals of 6–46 weeks. Actuarial survival for the entire group was 71.5% at 30 weeks but was better for patients with grade III tumors (64% at 33 weeks) than for those with grade IV tumors (29% at 30 weeks). Toxic effects consisted of mild to moderate nausea and vomiting in 17 patients, sensory peripheral neuropathy in five, and transient somnolence in three. These data must be considered only preliminary. Further investigation of misonidazole is under way.

The theoretic basis for the use of radiosensitizers and radiopotentiators is valid, but a definitive judgment on their effectiveness in enhancing chemotherapy for brain tumors must await the results of additional studies.

COMBINATION-AGENT CHEMOTHERAPY

Following their 1972 series, Gutin et al (84) reported on 48 patients with recurrent primary deep tumors (unbiopsied but presumably malignant) or metastatic neoplasms treated with a PCV combination at 4-week intervals. This was a well-controlled study: all patients were treated at 3 months after completion of radiation therapy to avoid effects of irradiation, doses of steroids were carefully monitored, and only patients with improvement indicated both by clinical tests and RN scans were considered to be responders. Those who showed improvement in one parameter or stability in both for at least 3 months were considered to have stable disease. Malignant gliomas, medulloblastomas, and unbiopsied deep masses all improved with PCV therapy; the overall response rate was 44% (or 60% with the inclusion of patients with stable disease). Although the response was significant (median duration of response: 9 months), this protocol was not markedly more effective than single-agent therapy with PCB or BCNU. A newer PCV protocol appears to be more effective and somewhat less toxic. Fifteen patients treated with PCV after the tumors had progressed following radiation therapy plus BCNU or radiation therapy plus HU and BCNU had a median time to progression of nearly 5 months.

Hildebrand et al (85,86) treated 22 recurrent primary tumors and 31 metastatic tumors with a combination of CCNU, VCR, and MTX with CVR, in a phase II study. A remission was defined as clinical improvement present 6 weeks after discontinuation of steroids and lasting ≥ 4 weeks. Patients who had had radiation therapy were not admitted to the study until at least 2 months had elapsed. Malignant gliomas responded clinically in four (25%) of 16 pa-

tients for a mean of 37 weeks. Although this response rate is less than that reported with PCV, the mean duration of response was approximately equal in each study. In five (46%) of 11 patients with metastatic breast tumors, remission lasted a median of 16 weeks. Metastatic lung carcinoma responded in only one (5%) of 20 patients. The relative resistance of lung carcinoma metastases to chemotherapy, especially to CCNU, has been observed in other studies (87). The most frequent toxic complication was thrombocytopenia. No deaths were attributed to drug toxicity.

Levin et al (88) reported on 65 patients (valid malignant glioma study group, 45 patients) treated with BCNU and PCB on a 6-week schedule. The parameters of treatment timing were: within 2 months after operation, at 3 months after irradiation, and at least 6 weeks after chemotherapy. Evaluation of response and stable disease was as described by Shapiro and Ausman (7). All evaluable patients had received at least two courses of chemotherapy, and dexamethasone (Decadron) usage was taken into consideration in evaluating the response. The combined response rate of 47% (21 of 45) for those patients with prior surgery and irradiation did not differ significantly from the 49% response rate (seven of 16) for patients without prior surgery or irradiation, nor was there a statistically significant difference in the median duration of response between the two groups. More than half of the patients had toxic manifestations, with myelosuppression severe enough to require dose reduction to nontherapeutic levels (ie, 25% of the original dose) in 15 (33%) of 45 patients. One patient developed severe myelotoxicity after two courses of therapy (300 wbcs/mm^3 and only 16,000 platelets/mm^3) and died during the seventh week of the study. The frequency and duration of response did not appear to correlate statistically with sex, age, duration of symptoms, or prior chemotherapy. The investigators concluded that there is no advantage in the combination of BCNU and PCB over the use of each agent alone and, moreover, that the combination was inferior to PCV for patients with recurrent malignant glioma.

In a phase II–III trial, Shapiro and Young (89) treated 33 patients who had malignant gliomas with either BCNU and VCR (group A, 16 patients) or BCNU, VCR, and 6000 rad of irradiation in 6 weeks (group B, 17 patients). The groups were randomly selected 2 weeks after surgery and were comparable in all respects except Karnofsky status (group A > group B). Although the median survival time of patients not receiving postoperative irradiation (30 weeks) was less than that of those with radiation therapy (44.5 weeks), this was not statistically significant by the Wilcoxon signed-ranks test. Toxic effects were mild, but one patient died of pulmonary fibrosis secondary to nitrosourea therapy.

Subsequently, Shapiro and Young (90) reported another phase II–III trial with CCNU at two dose schedules (130 mg/m^2 orally every 6 weeks or 80 mg/m^2 orally every 2 weeks). They found the schedule with doses every 2 weeks to be more effective in a small number of patients with recurrent malignant glioma. It was also more effective as an adjunct to surgery and irradiation. On the basis of this data, they treated 24 patients with CCNU and VCR on Days 1 and 15 of a 28-day cycle, and PCB on Days 1–28. In 17 patients who had surgery and postoperative irradiation with adjunctive PCV therapy, the projected median survival time was > 50 weeks. This exceeded prior BCNU-VCR protocol results of these investigators by approximately 15 weeks and was complementary to the results of the phase II studies by Fewer et al (49) and Gutin et al (84).

Taylor et al (26) treated 44 patients who had malignant gliomas with DTIC and/or a nitrosourea in a phase II–III protocol. It is difficult to evaluate the efficacy of DTIC in the phase II trial because only 14 patients were allocated into four treatment groups. In the phase III trial, patients who had chemotherapy received DTIC, MeCCNU, or a combination of both. It appears that chemotherapy, whether with MeCCNU, DTIC, or their combination, resulted in a mean survival time of 55 weeks, compared with 35 weeks for the control group. The major benefit was probably from the MeCCNU, and the combination of the DTIC and MeCCNU was more toxic and not significantly more effective than either drug used alone. Two patients who had combined therapy developed life-threatening sepsis secondary to myelosuppression. Only limited information on the effect of DTIC can be gained from this study.

In 1976, Frias-Monserrate et al (91) treated eight recurrent childhood brain tumors with mechlorethamine, VCR, prednisone, and PCB (MOPP) on the Hodgkin's schedule. All patients had end-stage disease; six were comatose. Four patients responded partially, and one patient, who was comatose, showed improvement in the RN scan as well as in clinical indications of neurologic status. Toxic side effects (nausea, vomiting, myelosuppression) were mild. Further investigation of this protocol should be considered.

Pouillart et al (92) reported on a phase II trial of doxorubicin in combination with VM-26 and CCNU in 43 patients with recurring or inoperable malignant gliomas and in 30 with metastatic tumors. VM-26 and CCNU were used because Pouillart had pre-

viously found a 60% improvement in neurologic status and a 6-month median survival time in a small number of patients. The reason for adding doxorubicin, which prior evidence suggests to be ineffective, is not reported. Response was defined as improvement in clinical status lasting for a minimum of 2 months. Changes in steroid levels were taken into account in evaluating response, but the results of this study are clouded by addition of an "antihypertensive regimen" (hypertonic glucose, mannitol, and furosemide) administered with each chemotherapy treatment. A surprising response rate of 72% (31 of 43 patients) is reported for patients with malignant gliomas, who had a median survival time of 26 weeks; but the time to progression is not reported. Clinical improvement was most frequently seen during the first 2 months of therapy. Absence of improvement after two courses was associated with a poor prognosis. As might be expected on the basis of other studies previously noted, metastatic lung carcinoma showed a poor response: one (8%) of 13 patients; whereas six (75%) of eight patients with metastatic breast tumors responded, with a median survival time of 35 weeks. Twenty percent of the improved patients simultaneously exhibited deterioration on RN scans.

Although the results are blurred by the addition of the antihypertensive treatment, it does not appear that doxorubicin was beneficial in this combination, and the combination of BCNU and VM-26 reportedly had a greater effect on the peripheral tumors of patients with metastatic cerebral lesions than on the cerebral lesions themselves.

In 1977, Mealey and Hall (93) reported the results of a phase II–III study of childhood medulloblastoma, in which 45 patients were treated with surgery and postoperative irradiation. Radical and subtotal resection apparently did not increase the surgical mortality rate, which was 11%. In the first 3 years, 47% of the patients died, and the continuous mortality after 10 years was 5%–10% per year up to 13 years after diagnosis: 53% were alive at 3 years, 41% at 5 years, and 22% at 10 years. Recurrent symptoms developed in 29% of the patients in the original series. The 11 children found to be free of disease had received more craniospinal irradiation than those who experienced recurrence ($P < 0.001$). Early recurrences, appearing an average of 12 months after surgery, were noted in 14 (63%) of 22 patients and were more frequently intracranial. Late recurrences, 2 years after surgery, were situated in the posterior fossa in six patients; the one other late recurrence in the posterior fossa was probably local. Eight children manifested postoperative spinal recurrences within a mean of < 7 months; there was no difference in the amount of spinal radiation they received. Of 22 children whose tumors (six intracranial and eight intraspinal) recurred in < 4 years, 14 (63%) were treated with supplemental irradiation and chemotherapy and survived an average of 10 months following recurrence; eight patients who did not receive additional irradiation or chemotherapy on recurrence survived < 2 months ($P \leq 0.001$). Patients whose early recurrences were intracranial survived longer (average, 22 months) than those who developed spinal metastases (average, 17 months).

Hendler et al (94) treated 35 patients with surgery and postoperative irradiation (6000 rad) and subsequent chemotherapy in a phase II–III study. After surgery, patients were randomized into two treatment groups (BCNU or BCNU and VM-26). The valid study group consisted of 19 patients; five (50%) of ten patients receiving BCNU alone responded clinically for a mean of 5+ months and seven (87%) of eight patients treated with the combination of VM-26 and BCNU responded clinically for a mean of 5+ months. Five patients who did not respond to BCNU were crossed over to VM-26, and three patients in this small group appeared to respond clinically. Toxic symptoms (prolonged leukopenia attributable to BCNU, and anaphylaxis and hepatotoxicity due to VM-26) were noted in four patients; no deaths related to toxicity were reported.

In a brief communication, Gutin and Walker (95) reported little effectiveness in the combination of VM-26 and MeCCNU used in a phase II study to treat 26 patients who had malignant glioma. Toxic effects consisted of delayed marrow suppression.

In a phase III trial, Bonadonna et al (96) randomized 50 patients with supratentorial malignant gliomas into three groups, 2 weeks after surgery. Steroids were not used during the period of chemotherapy. Response was reported as the percentage of recurrence in each group. Patients treated with an unspecified amount of irradiation had a 66% recurrence rate, while those treated with irradiation plus either BCNU or CCNU at unspecified dose and schedule had respective recurrence rates of 74% and 44%. These differences were not, however, statistically significant, and the populations were not matched statistically. Moreover, little can be determined on the basis of the recurrence rate alone, since almost all malignant gliomas will recur eventually.

In a report presented at the 1977 meeting of the International Society of Pediatric Oncology (SIOP) (Philadelphia, 21–24 September), Fossate-Bellani et al claimed a 4-year survival rate of 80% (83% at 3 years, 100% at 2 years) in 16 children with medulloblastoma who were treated with surgery fol-

lowed by irradiation (5000–5500 rad) of the CNS and posterior fossa. Immediately after surgery they were given intrathecal MTX and iv VCR. One month after completion of irradiation, a combination of BCNU, MTX, and VCR was started and continued for 2 years; in 1974, CCNU replaced BCNU. In 31% of the patients, some thrombocytopenia occurred, necessitating discontinuation of therapy.

Heiss (97) treated 67 patients who had primary malignant gliomas (grade III or IV) in a phase II–III trial. A control group of 16 patients had gross total or subtotal resection, and eight of these received postoperative irradiation. Combination drug therapy was given to 27 patients in two to four consecutive 7-day cycles as follows: PCB (125 mg iv) and 6-mercaptopurine (50 mg orally) daily; cyclophosphamide (300–500-mg iv infusion) on Days 1,3, and 5; 5-FU (250-mg iv infusion) on Days 2,4, and 6; and MTX (5 mg iv) on Day 7; all patients received daily methylprednisolone (20 mg iv) during therapy. Of the original 27 patients receiving combination therapy, 11 were treated at the time of recurrence (phase II), 11 after primary surgical resection, and five following a second operation. A group of 24 patients received CCNU (100 mg/m^2 orally every 6 weeks) beginning 2–3 weeks after primary surgical resection of their tumors. The control group exhibited a mean time of progression of 4.1 months (mean survival, 6.3 months) versus 5.4 months for 16 patients who received combination-drug therapy after surgery (mean survival, 9.0 months) and 5.2 months for 24 patients treated with CCNU (mean survival, 7.0 months). Eight of the 11 patients receiving combination therapy at the time of recurrence showed clinical improvement in neurologic status lasting 0.8–4.0 months. Patients in the group receiving postoperative combination-drug therapy exhibited a longer mean survival than those who had surgery alone ($P < 0.05$). Although survival time appears to have been better with combination therapy than with CCNU, this was not statistically significant. Combination therapy occasionally caused severe bone marrow suppression necessitating interruption of treatment.

In a subsequent study, Heiss et al (98) treated 22 patients who had primary malignant gliomas (grades III and IV) with a combination chemotherapy regimen consisting of CCNU, VCR, MTX, and PCB (COMP). All patients had surgery and histologic confirmation of tumors. Therapy was started 10–20 days after surgery; patients received CCNU (100 mg/m^2 orally) on Day 1, VCR (1.4 mg/m^2 iv) on Days 1 and 8, MTX (10 mg/m^2 im) on Days 3,7,10, and 14, and PCB (100 mg/m^2 iv) on Days 2–15. This 15-day cycle was repeated twice after 6-week intervals and every 3 months thereafter until recurrence of the tumor or death of the patient. During chemotherapy, all patients received steroids (20 mg/day of methylprednisolone). Patients receiving COMP therapy exhibited a median time to recurrence (as determined by neurologic examination or RN scan) of 8.9 months and a median survival time of 10.7 months. These results are significantly better ($P < 0.01$) than those for the control and CCNU groups in the previous study (97) but not better than those for the combination drug regimen in that study. Toxicity consisted of gastrointestinal irritation, myelosuppression, and neurotoxicity (secondary to VCR). In no instance was myelosuppression so severe that it was necessary to interrupt therapy, which suggests that COMP therapy is less toxic than the combination-drug therapy used in the earlier study.

Levin et al (99) have treated 29 malignant glioma patients at UCSF with BCNU (180 mg/m^2 on Day 1) in combination with 5-FU (1000 mg/m^2/day by continuous infusion for 3 days beginning on Day 14). Use of this combination was based on the theory that administration of a cell-cycle-specific drug (5-FU) during the period of active tumor repopulation following a single dose of BCNU should maximally reduce the proliferating tumor cell population and prevent cell proliferation for at least 4–5 days. Nine patients (31%) responded; improvement was indicated by both clinical neurologic examination and RN brain scan while the patient was given a stable or decreasing dose of dexamethasone. Median time to progression was 34 weeks. Fifteen patients (52%) were classified as having stable disease; stability was indicated by clinical neurologic examination or RN brain scan, with improvement, stability, or deterioration in the other parameter while steroid doses were stable or decreasing. The median time to progression was 25 weeks. Toxic effects consisted of cumulative myelosuppression. Patients classified as responders were found to have tolerated larger doses of BCNU (exhibiting less myelosuppression) and to have had a higher Karnofsky score (76 vs 56; $P \leq 0.0001$) at the start of therapy. The combination of BCNU and 5-FU has resulted in higher rates of cumulative response and stable disease than any other combination therapy used in studies at UCSF.

In 1978, Zander et al (100) reported preliminary results in seven patients treated with a combination of BCNU and VM-26. Six had recurrent gliomas following surgery and irradiation, and one had reticulum cell sarcoma with bone involvement. Four of the seven responded to therapy (criteria not specified); they survived a median of 7 months. These results are not significantly better than those obtained with BCNU as a single agent.

TABLE 1.—Single-agent chemotherapy

Author(s)	Treatment	Tumor type	Response*	Survival data† (wks)
Benjamin (1974)	Doxorubicin (Adriamycin)	Medulloblastoma	0/6	
Kanno (1970)	Bleomycin	Malignant glioma	6/12 (50%)	
Takeuchi (1975)	Bleomycin	Low-grade glioma	9/16 (56%)	1 yr, 50% 2 yrs, 35% 3 yrs, 22% 4 yrs, 14%
	Bleomycin	Malignant glioma	7/10 (70%)	
Eagan (1979)	Continuous radiotherapy	Malignant glioma or glioblastoma multiforme		Median ST: 37
	Split-course radiotherapy	Malignant glioma or glioblastoma multiforme		Median ST: 35
	Continuous radiotherapy + DAG	Malignant glioma or glioblastoma multiforme		Median ST: 68
	Split-course radiotherapy + DAG	Malignant glioma or glioblastoma multiforme		Median ST: 68
Skylansky (1974)	VM-26	Malignant glioma and metastasis	5/13 (38%)	Median DR (range): 15+ (13–61)
Khanna (1969)	Epodyl	Low-grade glioma (55 patients) Malignant glioma (24 patients)	46/79 (58%)	
Edland (1971)	Radiotherapy	Glioblastoma multiforme		Mean ST: 49
	Radiotherapy + 5-FU	Glioblastoma multiforme		Mean ST: 50
SWOG (1975)	BIC	Ependymoma	0/1	
Finklestein (CCSG) (1975)	DTIC	Medulloblastoma	0/1	
Levin (1975)	BIC	Malignant glioma	4/19 (21%)	
Weiss (1969)	MTX	Glioblastoma multiforme	4/7 (57%)	ST range: 6–21
Garfield (1973)	MTX	Malignant glioma + metastasis		Mean ST (range): 20.3 (15–56)
Djerassi (1977)	MTX	Malignant glioma (child)	6/7 (86%)	ST range: 9–109+
Rosen (1977)	MTX	Brain stem glioma	2/4 (50%)	ST: 26 and 78
	MTX	Medulloblastoma (child)	5/7 (71%)	Median DR: 40
Shapiro (1977)	MTX	Malignant glioma	0/6	
Mealey (1970)	Mithramycin	Malignant glioma	0/3	ST range: 0.4–17
Walker (BTSG) (1976)	Mithramycin	Malignant glioma	52	Median ST: 21
	Controls	Malignant glioma	44	Median ST: 26
Owens (1969)	Mechlorethamine	Glioblastoma multiforme	0/15	
	Mechlorethamine	Metastasis	0/6	
Broder (1970)	BCNU	Malignant glioma	2/4 (50%)	
Walker (1970)	BCNU	Low-grade glioma	10/16 (62%)	DR range: 13–77
Wilson (1970)	BCNU	Glioblastoma multiforme	7/18 (39%)	
		Malignant glioma	4/6 (66%)	
Armentrout (1974)	Surgery + radiotherapy	Malignant glioma	11	Median ST: 30; 1 yr, 14%
	Surgery + radiotherapy + BCNU	Malignant glioma	16	Median ST: 43; 1 yr, 55%

			VSG‡	*ATG*‡	*18 mos*
Walker BTSG (1978)	BCNU	Malignant glioma	18.5%	25.0%	0
	Radiotherapy	Malignant glioma	36.0%	37.5%	4%
	Radiotherapy + BCNU	Malignant glioma	34.5%	40.5%	19%
	Best care	Malignant glioma	14.0%	17.0%	0

Hansen (1971)	CCNU	Malignant glioma	5/6 (83%)	Mean DR (range): 24 (13–52)

TABLE 1.—Single-agent chemotherapy—Continued

Author(s)	Treatment	Tumor type	Response*	Survival data† (wks)
Fewer (1972)	CCNU (prior BCNU)	Malignant glioma or metastasis	0/8	ST range: 4–30
	CCNU (no prior BCNU)	Malignant glioma or metastasis	7/21 (33%)	
Rosenblum (1973)	CCNU	Malignant glioma	43%	Median DR: 22
	CCNU	Metastasis	37%	
Hoogstraten (1973)	CCNU	Malignant glioma	5/14 (36%)	DR range: 4–19
				Median DR / *Median ST*
Weir (1976)	Radiotherapy	Malignant glioma	15	23§ / 27
	CCNU	Malignant glioma	13	14§ / 37
	Radiotherapy + CCNU	Malignant glioma	13	31 / 36
Reagan (1976)	Radiotherapy	Malignant glioma	22	Median ST: 50
	CCNU	Malignant glioma	22	Median ST: 28
	CCNU + radiotherapy	Malignant glioma	19	Median ST: 52
EORTC (1978)	Radiotherapy + late CCNU	Malignant glioma or glioblastoma multiforme	42	Mean ST: 62 from surgery (22 from relapse) Mean DF: 31
	Radiotherapy + early CCNU	Malignant glioma or glioblastoma multiforme	39	Mean ST: 43 from surgery (11 from relapse) Mean DF: 34
	Radiotherapy	Malignant glioma or glioblastoma multiforme	11	Mean ST: 22
	Radiotherapy + CCNU	Malignant glioma or glioblastoma multiforme	8	Mean ST: 32
Young (1973)	MeCCNU	Malignant glioma	2/15 (13%)	DR: 22
Tranum (SWOG) (1975)	MeCCNU	Malignant glioma	8/29 (28%)	
Walker (BTSG) (1978)	MeCCNU	Malignant glioma		Median ST: 31
	Radiotherapy	Malignant glioma		Median ST: 36
	Radiotherapy + MeCCNU	Malignant glioma		Median ST: 34.6
	Radiotherapy + BCNU	Malignant glioma		Median ST: 50.3
Brisman (1976)	Various nitrosoureas	Malignant glioma or glioblastoma multiforme		
				Median ST:
	Group A			
	Chemotherapy			52
	No chemotherapy			18
	Group B			
	Chemotherapy			52
	No chemotherapy			39
	Group C			
	Chemotherapy			26
	No chemotherapy			27
Kumar (1974)	PCB	Malignant glioma + metastasis	12/29 (41%)	Median DR: 30
Edwards (1979)	ThioTEPA	Malignant glioma	0/12	
Owens (1969)	VCR	Malignant glioma	30	Mean ST: 61
Smart (1968)	VCR	Various	8/16 (50%)	
Braham (1969)	VCR	Malignant glioma	4/4 (100%)	DR range: 63–78
Afra (1973)	VCR	Malignant glioma	7/9 (78%)	Mean ST: 27
Rosenstock (1976)	VCR	Various	8/16 (50%)	Median DR: 125

*No. of patients responding/No. treated; otherwise, No. of patients treated.
†ST = survival time; DR = duration of response; DF = disease-free interval.
‡VSG = valid study group; ATG = patients receiving > 5000 R, ≥ 2 courses of BCNU, minimum guaranteed survival of 8 wks.
§Median time to crossover.

Crafts et al (101) recently reported the use of PCV in treating recurrent medulloblastomas. Two treatment schedules were used. PCV_1 consisted of PCB (100 mg/m² orally) on Days 1–14, CCNU (75 mg/m² orally) on Day 1, and VCR (1.4 mg/m² iv) on Days 1 and 8. PCV_2 differed only in delaying the course of PCB until Days 8–21. All patients had had suboccipital craniectomy and partial or gross total resection of tumor; in addition, all had received craniospinal irradiation in the postoperative period. Chemotherapy for tumor recurrence was started 10 months to 4½ years after completion of radiation therapy.

Of the 16 recurrent tumors treated, ten showed a response (as determined by subjective evaluation of neurologic improvement as well as radiographic evidence of decrease in tumor size), and four showed stability of disease progression (no change in subjectively determined neurologic status or tumor size). The median time to progression was 45 weeks. The two schedules tested were equally effective, but PCV_1 caused less myelotoxicity. Treatment with myelotoxic agents such as CCNU and PCB was severely compromised by diminished bone marrow reserve that resulted from prior craniospinal irradiation. After an initial response, there was frequently deterioration following reduction of the dosages of CCNU and PCB due to myelotoxicity. The results suggest that PCV has significant short-term effectiveness against recurrent medulloblastoma.

In 1979, Duffner et al (102) reported on the use of combination chemotherapy to treat five children with recurrent medulloblastoma. Therapy consisted of an induction period (5 weeks), in which VCR (2 mg/m²) was given once a week with concurrent intrathecal MTX (12 mg/m²/week) and dexamethasone (8 mg/m²/day). At the conclusion of the induction period, the patients received MTX (500 mg/m² iv) over 24 hours, with simultaneous intrathecal MTX (12 mg/m²), followed by VCR 24 hours later. MTX was administered both iv and intrathecally weekly on three subsequent occasions. All patients received maintenance therapy consisting of monthly BCNU (100 mg/m²), VCR (2 mg/m²), and dexamethasone (8 mg/m²) for 2 years. All five children responded for periods of 19–48 months. Two are alive without evidence of recurrence at 30 and 48 months; in three patients the disease recurred at 18, 19, and 28 months. One patient received concurrent low-dose irradiation at the time of recurrence. Toxic effects were reported to be mild, but specific levels of myelosuppression were not stated. As in previous studies, medulloblastoma seemed to be responsive to combination chemotherapy, but additional patients must be treated before a firm conclusion can be drawn.

The addition of CCNU, PCB, and bleomycin after surgery and before radiation therapy, with subsequent maintenance therapy consisting of CCNU, was evaluated by Seiler et al (103). The study group comprised 22 consecutive patients who had maximally feasible tumor removal of a histologically confirmed supratentorial glioma of grade III or IV; this group was compared with 32 historic controls with similar histology. Both groups received 6000–6800 rad to the tumor volume, although the technique of administration varied between the groups. Apparently, steroids were used in equal amounts in each group for 10–14 days following surgery. Induction chemotherapy began the second week following surgery, with CCNU (75 mg/m² orally) on Day 1, followed by PCB (100 mg/m² orally) once a day for 2 weeks. Radiation therapy was begun during the second week of PCB (the third week following surgery) and continued for 7 weeks; during the course of radiation, bleomycin (15 mg/m² im) was given three times per week for 5 weeks. The cycle of CCNU and PCB was repeated during the last week of radiation therapy. Maintenance chemotherapy consisted of CCNU (130 mg/m² orally, adjusted for myelotoxicity) beginning 4 weeks after the end of the second course of PCB and continued at intervals of 6–8 weeks for up to 1 year if the patient's neurologic examinations and RN scans indicated stable disease.

Bleomycin produced severe toxicity, necessitating discontinuation in eight cases (two patients with hyperthermia, six with skin reaction). No deaths due to toxicity were reported. The median survival time was 56 weeks for patients treated with chemotherapy and 51 weeks for the historic controls. At 76 weeks following surgery, eight (40%) of the treated patients were alive, versus nine (28%) of the controls. These differences were not statistically significant. It appears, therefore, that CCNU and bleomycin used as an adjunct to radiation therapy had no significant effect on survival.

In a more recent phase II study, Seiler et al (104) used a combination regimen of VM-26 and CCNU, with steroids, to treat 27 patients who had inoperable or recurrent malignant brain tumors (21 supratentorial astrocytomas, four brain stem gliomas, one medulloblastoma, and one supratentorial ependymoma). The majority had had surgery (18 patients) and irradiation (20 patients), and four had had previous chemotherapy. All received prednisone (100–200 mg/day, tapered to 12.5 mg every other day) and, following stabilization of neurologic status, VM-26 (120 mg/m² iv every 4 weeks) and CCNU (first cycle, 120 mg/m² orally; subsequent cycles, 80 mg/m²). After three full courses of this regimen, response was evaluated on the basis of

TABLE 2.—Radiosensitizers and radiopotentiators

Author	Treatment	Tumor type	No. of patients treated	Survival data*		
				1 yr	*2 yrs*	*3 yrs*
Sano (1972)	BUdR	Highly malignant glioma	37	59%	36%	15%
	BUdR	Moderately malignant glioma	25	90%	82%	64%
Lerner (1975)	HU	Malignant glioma	15	56 wks		
Irwin (1975)	Placebo + radiotherapy	Malignant glioma	14	34 wks		
	HU + radiotherapy	Malignant glioma	15	52 wks		
Urtasun (1976)	Radiotherapy	Malignant glioma	16	15 wks		
	Radiotherapy + metronidazole	Malignant glioma	15	26 wks		
Carabell (1979)	Radiotherapy + misonidazole	Malignant glioma or glioblastoma multiforme	41	72% at 30 wks (actuarial)		

*Values = median unless otherwise indicated.

prednisone dose, CT or RN scan, and neurologic examination. There were three complete and 12 partial responses for an overall response rate of 55% (15 of 27); the duration of response was 17–104 weeks, averaging 58 weeks. Skin allergy necessitated discontinuation of VM-26 for three patients. Myelosuppression secondary to CCNU resulted in treatment delays on 11 occasions and one death from thrombocytopenia. A similar response rate (72%) was reported by Pouillart et al (92) using doxorubicin, VM-26, and CCNU, but Gutin and Walker (95) reported little effect from the use of VM-26 in combination with MeCCNU.

The most recent randomized, prospective phase III trial is that of Levin et al (105), in which the effectiveness of BCNU, HU, and radiation therapy in combination (BHR) was compared with a combination of BCNU and radiation therapy (BR) for the treatment of 133 patients who had supratentorial malignant gliomas. The valid study group was comprised of 99 patients, stratified according to histologic type and extent of tumor resection. Within 4 weeks of surgical confirmation or unequivocal clinical and neuroradiologic diagnosis, BCNU (80 mg/m^2 iv) was administered to both treatment groups for 3 days; on Day 4, radiation therapy was begun (5000 rad to the whole brain and a boost of 1000 rad to the tumor area administered at 170–200 rad/day); the BHR group received HU (275 mg/m^2 every 6 hours) on alternate days from Day 5 until completion of radiation therapy. BCNU was given again for 3 days, beginning 2 weeks after completion of radiation therapy and repeated at intervals of approximately 8 weeks, until there were signs of tumor progression; the dosage was adjusted for myelotoxicity. BCNU produced insignificant gastrointestinal irritation, but HU was tolerated without ill effects by only 15 of 55 patients receiving it. Response to the therapy was determined by the parameters previously described by Levin et al (8). Karnofsky performance status (PS) was evaluated at the time of randomization and prior to each treatment.

For patients with glioblastoma multiforme, the median time to progression did not differ significantly by the Gehan-Wilcoxon analysis ($P = 0.04$) in the two treatment groups: 42 weeks for the BHR group, and 31 weeks for the BR group. This difference does, however, become significant ($P < 0.05$ by Cox analysis) when the dose of HU actually received by each patient is considered. For patients with malignant gliomas other than glioblastoma multiforme (NGM), there was no difference between the two treatment groups: 50 weeks for the BHR group, and 73 weeks for the BR group. There was, however, a significant difference ($P = 0.03$ by Gehan-Wilcoxon analysis) among the patients with glioblastoma multiforme who had a Karnofsky PS > 60 and had total or subtotal resection: the median time to progression was 41 weeks for the BHR group and 31 weeks for the BR group. No similar benefit was found for patients with NGM. Moreover, when all patients with glioblastoma multiforme were considered, irrespective of PS rating, the response was similar in both of these groups and for the NGM group.

The most significant finding is that the median time to progression for patients with glioblastoma multiforme was longer for those who received HU (42 weeks) than for those who did not (31 weeks). For patients with glioblastoma multiforme who received HU, the addition of subtotal or total resection resulted in a 17-week increase in the median time to progression (49 weeks); however, the ad-

TABLE 3.—Combination-agent chemotherapy

Authors	Treatment	Tumor type	Response*	Survival data* (wks)
Gutin (1975)	PCV	Glioblastoma multiforme	3/9 (33%)	Median DR: 39
	PCV	Malignant glioma	14/21 (66%)	
				Mean DR *Mean ST*
Hildebrand (1973, 1975)	CCNU + VCR + MTX	Malignant glioma	4/16 (25%)	37 53
	CCNU + VCR + MTX	Low-grade glioma	4/6 (66%)	41 69
	CCNU + VCR + MTX	Metastatic (breast)	5/11 (46%)	16 35
	CCNU + VCR + MTX	Metastatic (lung)	1/20 (5%)	80 92
Levin (1976)	BCNU + PCB (no prior surgery or radiotherapy)	Malignant glioma	5 R + 2 SD/16 (49%)†	Median DR: 20
	BCNU + PCB (prior surgery + radiotherapy)	Malignant glioma	13 R + 8 SD/45 (47%)†	Median DR: 34
Shapiro (1976)	Surgery + BCNU + VCR	Malignant glioma		Mean ST: 30
	Surgery + radiotherapy + BCNU + VCR	Malignant glioma		Mean ST: 44.5
Shapiro (1976)	CCNU	Malignant glioma (recurrent)	5	DR range: 10–26
	Surgery + radiotherapy	Malignant glioma	5	DR range: 108–211+
	CCNU + VCR + PCB	Malignant glioma (recurrent)	5/7 (71%) died 2/7 (29%) alive	ST range: 6–80 DR range: 46–82
	Surgery + radiotherapy	Malignant glioma	8/17 (47%) died 9/17 (53%) alive	ST range: 13–51 DR range: 29–82
				Mean DR *Mean ST*
Taylor (1975)	DTIC	Malignant glioma	5/8 (62%)	18 38
	DTIC + CCNU or MeCCNU	Malignant glioma	3/16 (19%)	12 32
	DTIC or MeCCNU	Malignant glioma	2	47 55
	DTIC + MeCCNU	Malignant glioma	6	59
	Surgery + radiotherapy	Malignant glioma		35
Frias-Monserrate (1976)	MOPP (Hodgkin's schedule)	Brain tumor (child)	4/8 (50%)	Mean ST: 17
Pouillart (1976)	Doxorubicin, VM–26, CCNU	Malignant glioma	31/43 (72%)	Median ST: 26
	Doxorubicin, VM–26, CCNU	Metastatic (lung)	1/13 (8%)	ST: 10
	Doxorubicin, VM–26, CCNU	Metastatic (breast)	6/8 (75%)	Median ST: 35
				3 yrs *5 yrs* *10 yrs*
Mealey (1977)	Surgery + radiotherapy	Medulloblastoma	32	53% 41% 22%
	Radiotherapy + VCR, MTX, BCNU, or MeCCNU	Medulloblastoma (recurrent)	5/12 (41%)	Mean DR: 43 ST range: 9–100
	Radiotherapy	Medulloblastoma (recurrent)	12/29 (41%)	
Hendler (1977)	Surgery + radiotherapy + BCNU	Malignant glioma	5/10 (50%)	Mean DR: 22
	BCNU + VM–26	Malignant glioma	7/8 (88%)	Mean DR: 22
	VM–26‡	Malignant glioma	3/5 (60%)	
Gutin (1977)	MeCCNU + VM–26	Malignant glioma	0/23	

TABLE 3.—Combination-agent chemotherapy—Continued

Authors	Treatment	Tumor type	Response*	Survival data* (wks)
				Recurrence
Bonnadonna (1977)	Radiotherapy	Malignant glioma		66%
	Radiotherapy + BCNU	Malignant glioma		74%
	Radiotherapy + CCNU	Malignant glioma		44%
				2 yrs *3 yrs* *4 yrs*
Fossate-Bellani (SIOP) (1977)	Surgery + radiotherapy + MTX + VCR + BCNU	Medulloblastoma	16	100% 83% 80%
				Mean DR *Mean ST*
Heiss (1978)	Surgery	Malignant glioma	16	18 27
	Methylhydrazine + cyclophosphamide, 5-FU + MTX + methylprednisolone	Malignant glioma (postoperative, recurrent)	27	23 38
	CCNU	Malignant glioma (postoperative)	24	22 28
	COMP	Malignant glioma (postoperative)	22	36 43
Levin (1978)	BCNU + 5-FU	Malignant glioma	9/29 (31%) 15 SD/29 (52%)	Median DR: 34 Median DR: 25
Zander (1978)	VM–26 + BCNU	Malignant glioma	4/7 (57%)	Median ST: 30
Crafts (1978)	CCNU + PCB + VCR	Medulloblastoma	10/16 (63%)	Median DR: 45
Duffner (1979)	VCR + MTX + BCNU	Medulloblastoma	5/5 (100%)	Median DR: 121
Seiler (1978)	Radiotherapy (historic controls)	Malignant glioma or glioblastoma multiforme	32	Median ST: 51
	CCNU + PCB + bleomycin + radiotherapy	Malignant glioma or glioblastoma multiforme	22	Median ST: 56
Seiler (1979)	Prednisone + VM–26 + CCNU	Malignant glioma or medulloblastoma	15/27 (55%)	Median DR: 58
Levin (1979)	BCNU + HU + radiotherapy	Glioblastoma multiforme	27	Median DR: 42
	BCNU + radiotherapy	Glioblastoma multiforme	21	Median DR: 31
	BCNU + HU + radiotherapy	NGM	14	Median DR: 50
	BCNU + radiotherapy	NGM	18	Median DR: 73

*See table 1 for explanation.
†R = response; SD = stable disease.
‡Patients who did not respond to BCNU were crossed-over to VM–26.

dition of HU did not show any benefit in the NGM patients. The effectiveness of HU (which is cell-cycle specific) and BCNU (which is cell-cycle non-specific) against glioblastoma multiforme may be due to the higher labeling index in this tumor or to the ability of these drugs to synchronize cell cycles so that radiation-induced cell kill is enhanced.

CONCLUSIONS

1. Extensive investigations of chemotherapeutic agents have been carried out, but there are very few well-controlled phase II or III trials. Moreover, variation from series to series in the criteria used to determine response makes comparison of reported antitumor activity extremely difficult. In the future, all phase II drug trials should use specific and consistent criteria, including results of neurologic examination, RN scan, CT scan, and Karnofsky PS.

2. Although Vick et al (106) have recently re-emphasized the lack of a blood-brain barrier within a tumor, those drugs to which the blood-brain barrier is thought to be impermeable have shown only nom-

inal efficacy. It is possible that with different scheduling, these drugs may show greater effectiveness; but for the present, it appears that those drugs of small molecular size (< 450 mm) and high lipid solubility are the most effective.

3. In phase II studies, single-agent chemotherapy using cell-cycle-specific drugs has been disappointing. This is most likely due to the low growth fraction seen in most cerebral neoplasms. The usefulness of these drugs and their appropriate scheduling in conjunction with cell-cycle-nonspecific drugs are yet to be determined.

4. Cell-cycle-nonspecific agents, such as BCNU, CCNU, and PCB, have produced the highest response rates when used as single agents. Combination chemotherapy with PCV, BCNU + 5-FU, and VCR + MTX + BCNU has also shown significant antitumor activity. Further improvement in scheduling may increase the effectiveness of these combinations and decrease their toxicity.

5. Medulloblastoma has proved to be an extremely sensitive tumor, responsive to a large number of single agents (BCNU, VCR, MTX, PCB, and VM-26) as well as combination chemotherapy (MOPP, PCV, and BCNU + MTX + VCR).

6. In phase III trials, irradiation and chemotherapy together have been only slightly more effective than irradiation alone. Unfortunately, irradiation followed by BCNU continues to be the standard treatment with which all other therapies must be compared.

7. There is need for phase II evaluations of other drugs and new combinations and dosage schedules. Perhaps the success of therapy will be improved by new radiation sources such as high-energy proton and neutron beams, more novel radiation therapy fractionations, and/or the use of radiosensitizing agents. All of these improvements and more will be necessary to improve on the disappointing results thus far obtained.

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Toxicity of Spirogermanium in Mice and Dogs After Iv or Im Administration[1,2]

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SUMMARY

Toxicity of single-dose spirogermanium was evaluated after iv and im administration to CDF_1 mice and beagle dogs. The im LD50 in mice was approximately threefold greater than the iv LD50. The lethal dose in dogs was the same for both routes of administration, but death was delayed after im injection. Convulsive seizures occurred only after the im doses that were lethal, but they were observed after administration of iv doses that were nonlethal. Microscopic evidence of drug toxicity (necrosis and degeneration) was found in mitotically active tissues: intestinal tract, lymphoid tissue, and bone marrow. Necrosis, hemorrhage, edema, and granulation tissue were observed in the muscle injection site.

[Cancer Treat Rep 64:1207–1210, 1980]

Spirogermanium is an azaspiran antineoplastic agent first synthesized and characterized in 1974 by Rice et al (1). These investigations demonstrated that spirogermanium was cytotoxic against HeLa and human malignant lymphoma cells in vitro and was active against L1210 leukemia cells in tissue culture. They also showed that protein synthesis was inhibited by this agent. Antitumor activity has been demonstrated for spirogermanium in several experimental tumor test systems including Walker 256 carcinosarcoma, 13762 mammary adenocarcinoma, and 11095 prostatic carcinoma.[6] Phase I clinical trials have utilized both im and iv routes of administration, and the present study was conducted to compare the toxicity of single-dose spirogermanium when administered by these routes to mice and dogs.

MATERIALS AND METHODS

Purebred beagle dogs were supplied by the Animal Genetics and Production Branch of the National Cancer Institute (Bethesda, MD), through the Battelle Toxicology Program Office. They were housed in individual steel cages, and food and water were available ad libitum. At least two complete hematologic and serum chemistry determinations were performed for each dog before treatment. Blood was obtained from treated animals twice during the first week and once weekly during the 45-day observation period. Dogs were treated with spirogermanium in pairs, one male and one female, at each dose level. The preclinical toxicologic evaluation included physical examinations, hematologic and serum chemistry determinations, necropsies, and histopathologic evaluations.

CDF_1 mice were received from the Animal Genetics and Production Branch and maintained on hardwood bedding with food and water available ad libitum. Each dose of spirogermanium was administered to 13 males and 13 females, with three animals per sex serving as satellite groups. The remaining ten animals per sex were used for LD50

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TABLE 1.—Defined toxicity levels of spirogermanium in mice and dogs

Species	Toxicity	Route of administration and dose			
		Iv		Im	
		mg/kg	mg/m²	mg/kg	mg/m²
Mouse	LD90	48.60	145.8	163.1	489.3
	LD50	42.80	128.4	133.7	401.1
	LD10	37.70	113.1	109.6	328.8
Dog	LD	40.00	800.0	40.0	800.0
	TDH	20.00	400.0	20.0	400.0
	TDL	1.25	25.0	5.0	100.0
	HNTD	0.625	12.5	2.5	50.0

determinations. If an animal died unobserved during the 14-day observation period, an animal from the satellite group was necropsied at that time. Selected organs (liver, kidney, lung, duodenum, heart, spleen, pancreas, brain, and muscle injection site) were taken from three mice per sex per dose at the end of the observation period for histopathologic examination.

The chemical identity of spirogermanium[7] was verified by infrared spectroscopy and melting point determination. The drug was stored at 4°C in the dark. The drug, in a 5% aqueous solution, was found to be stable after 5 days at room temperature. The solubility, in 0.9% NaCl, was determined to > 200 mg/ml. For iv and im injections in dogs, concentrations of 25 and 200 mg/ml, respectively, in 0.9% NaCl were prepared; for mice, the corresponding concentrations were 2 and 25 mg/ml. The maximum volumes for im injection were 0.12 ml for mice and 4.0 ml for dogs.

The studies were performed and levels of toxicity (table 1) were determined according to the procedures described by Prieur et al (2) as modified by the Battelle Toxicology Program Office.[8]

The following definitions of dose levels were used in this study:

Highest nontoxic dose (HNTD)—the highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose produces the aforementioned alterations.

Toxic dose low (TDL)—the lowest dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces no lethality.

Toxic dose high (TDH)—the dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality.

Lethal dose (LD)—the lowest dose which produces drug-induced death in any animal during the treatment or observation period.

RESULTS

Iv Administration

Susceptibility to the toxic effects of spirogermanium in mice was similar for both sexes. The LD50 for females was 41.5 mg/kg (124.5 mg/m²) compared with 44.5 (133.5 mg/m²) for males. The majority of deaths for both sexes occurred on Day 1; a few females died 2 and 4 days after treatment. Toxic manifestations at LDs included tonic and clonic convulsions beginning immediately after treatment, dyspnea, hyperactivity, straub tail (vertical hypertension of the tail), bloody exudate from nose, and weight loss. Treated mice also appeared to be hypersensitive to normal background sound generated by technical support personnel. Microscopic lesions were limited to focal necrosis of lymphocytes in splenic follicles.

Seven pairs of dogs were injected iv with single doses of spirogermanium, one pair at each of the following levels: 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/kg (800, 400, 200, 100, 50, 25, and 12.5 mg/m²). At the highest dose level, the female died immediately after treatment, with toxic signs of tremors and apnea. The male had tonic and clonic convulsions during and immediately after the end of treatment, piloerection, mydriasis, excessive salivation, ataxia, uncoordinated eye movements, dyspnea, vocalization, tremors, and no response to external stimuli. Two hours after treatment, heartbeat was arrhythmic and the animal was cyanotic.

[7]Spirogermanium was supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[8]Procedures Manual, Battelle Columbus Laboratories, Feb 5, 1975.

Tonic and clonic convulsive seizures also occurred in both dogs treated with 20 mg/kg and in the female treated with 10 mg/kg. Other toxic manifestations in these dogs were similar but less severe than those observed at the highest dose. Weight loss and reduced food consumption were present at dose levels > 2.5 mg/kg.

Changes in hematologic and serum chemistry values were most severe at the 40-mg/kg dose level: hemoconcentration; lymphocytosis; nucleated rbcs; marked increases in BUN, alkaline phosphatase (AP), SGOT, SGPT, and calcium and magnesium ion levels; slight increase in creatinine and bilirubin (total and direct) levels; and decreased values for blood sugar levels. After treatment with 20 mg/kg, slight increases in SGOT levels and a marginal decrease in rbc count occurred during the first week; leukocytosis and increased AP levels were present 2–3 weeks after treatment. Quantitative changes in blood values were not strictly dose-related at lower dose levels. Although there were no significant changes at 10 mg/kg, the female treated with 5 mg/kg had decreases in hematocrit, hemoglobin, and rbc count values and had neutropenia, lymphocytosis, and hematuria. This differential shift occurred in the absence of any drug-related effect on total wbc count. The male had a moderate reduction in rbc count and a marginal increase in the SGPT level. A moderate increase in BUN value occurred in the female at the 2.5-mg/kg dose level; the male treated with 1.25 mg/kg had a slight decrease in rbc and wbc counts and a marked increase in SGPT value.

Severe mucosal lesions were evident in the small intestines of dogs given 40 mg/kg. Lesions in the small intestine were characterized by markedly shortened villi, necrosis of villus tips, necrotic crypt epithelium, severe congestion, and focal hemorrhage. Lymphoid depletion, hemosiderosis and erythrophagocytosis in lymph nodes, and an increased myeloid:erythroid ratio in bone marrow were also observed at the highest dose level. Intestinal changes were not observed at lower dose levels. Focal necrosis, focal hemorrhage, and hemosiderosis and erythrophagocytosis of lymphoid tissue were present to a mild degree at levels down to 0.625 mg/kg.

Im Administration

Toxic signs after single im injections into the vastus femoris muscle of mice were similar to those observed after iv treatment, but onset of the convulsive seizures was delayed for 5–10 minutes after treatment. Spirogermanium was less toxic to mice by the im route. The LD50 for female mice was 119 mg/kg (357 mg/m^2) compared with 142.9 mg/kg (428.7 mg/m^2) for males. Mice died 2–6 days after treatment, with the greatest number of deaths occurring on Day 2. In animals dying within 6 days, the musculature of the injection site showed severe focal necrosis, acute inflammatory infiltrate, focal hemorrhage, and edema. Vacuolation of hepatocytes was also observed. Microscopic lesions in mice necropsied 14 days after treatment included mineralization of the muscle bundles at the injection site, with a round cell infiltrate and fibrosis, vacuolation of hepatocytes, and focal necrosis in splenic lymphoid follicles.

Six pairs of dogs were used in the im study. They were treated with a single im injection of 80, 40, 20, 10, 5, or 2.5 mg/kg (1600, 800, 400, 200, 100, or 50 mg/m^2). At the 80-mg/kg dose level, tonic and clonic convulsive seizures occurred 5–15 minutes after treatment, and intermittent seizures were evident until death. Other clinical signs were similar to those observed at the iv LD. The male died within 1 hour; the female was killed in a moribund state 2.5 hours after treatment. Convulsive seizures, which were intermittent over a 4-hour period, occurred in the male treated with 40 mg/kg. The dog died within 18 hours after treatment. The female at this dose level had a brief episode of convulsive seizures 1 hour after treatment; reduced food consumption and weight loss were also observed. The only effects seen at 20 mg/kg were reduced food consumption and weight loss.

Clinical pathologic changes were similar to those observed after the iv route and were also most severe at the highest dose level. Increased values for renal function tests, BUN, creatinine, hemoconcentration, and leukocytosis with a differential shift occurred only at the 80-mg/kg dose level. Decreases in the hematocrit, hemoglobin, and rbc count values were observed at doses from 10 to 40 mg/kg. Leukocytosis (neutrocytosis) occurred at dose levels between 5 and 20 mg/kg. Quantitative changes in hepatic function tests and increases in AP, SGOT, and SGPT values were dose-related after im administration. Changes in hematologic and serum chemistry values were not evident in dogs treated with 2.5 mg/kg.

Hemorrhages were observed at gross examination and microscopically in a number of tissues from dogs that died after treatment with either 40 or 80 mg/kg. Lesions in the small intestine and colon in dogs treated at the highest dose included severe diffuse epithelial necrosis, focal hemorrhages, and acute inflammatory infiltrate. These microscopic lesions were also present in the muscle of the injection site at doses > 10 mg/kg. Shortened villi in the jejunal mucosa were present in the dog treated with 40 mg/kg and killed 45 days after treatment. Focal

necrosis and hyperplasia were observed in lymphoid tissue from dogs killed within 1 week after treatment at all doses. After a 45-day observation period, lymphoid hyperplasia was still evident. Granulation of tissue was present in the muscle of the injection site at this time.

DISCUSSION

Toxicosis attributable to spirogermanium was manifest in the central nervous system, hematopoietic and reticuloendothelial systems, intestinal tract, and liver. Values for renal function tests were increased only at LDs without microscopic evidence of tissue damage. The most prominent toxic sign was convulsive seizures, which occurred in mice and dogs after both routes of drug administration. In mice, hyperacusis may have been apparent after treatment. Populations of rapidly dividing cells (ie, bone marrow, gastrointestinal mucosa, and lymphoid tissue) were most severely affected by the higher dose levels. Although hematopoietic and intestinal toxic effects were reversible, lymphoid atrophy and hyperplasia were still evident in dogs 45 days after treatment.

Changes in liver function were evident by increased activities of AP, SGOT, and SGPT, but microscopic lesions were not observed in dog livers. Moderate to severe cytoplasmic vacuolation of hepatocytes occurred in mice treated by the im route. Quantitative changes in serum enzyme values in dogs were dose-related after im injection but not after iv treatment. Below the TDH established in the iv study, there was individual animal susceptibility to drug hepatotoxicity.

Peripheral wbc counts were elevated 2–3 weeks after treatment with spirogermanium by either the im or iv route. Within a few days after treatment, differential shifts were observed; however, total wbc counts were within normal limits. Although spirogermanium did decrease many of the rbc parameters (eg, hemoglobin, hematocrit, and rbc values), leukopenia was not encountered. Therefore, elevated myeloid:erythroid ratios probably reflected the cytotoxic effects of spirogermanium on erythrocyte precursors.

The results of these studies suggest that the route of administration affects the onset of spirogermanium toxicity. There are marked species differences in susceptibility to its lethal effects. Im injection markedly reduces lethality in mice relative to iv administration, whereas the LD for dogs is the same for both iv and im routes. Death was delayed, however, after im injection. Convulsive seizures occurred in dogs given iv doses below the LD level but were associated only with the LD after im injection. Dogs were more tolerant to im doses at the TDL and HNTD than to iv doses (table 1). Im injection also produced marked damage to the muscle of the injection site. Mice and dogs necropsied within 1 week after treatment showed severe focal necrosis, hemorrhage, and edema in the injected muscle. Marked increases in SGOT and neutrocytosis that occurred in dogs probably reflect muscle damage and an inflammatory response.

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Preclinical Toxicologic Evaluation of ICRF-187 in Dogs[1,2,3]

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SUMMARY

Single- and multiple-treatment schedules in dogs were used to evaluate the toxicity of ICRF-187. The major target organs were the bone marrow, lymphoid tissue, gastrointestinal tract, liver, and kidney. Liver, kidney, and intestinal toxic effects were most severe at lethal doses. Toxic effects on the kidney and intestinal tract were reduced or absent at lower doses. Hepatotoxicity was most severe after single doses and was only slowly reversible at high doses. Repeated dosage regimens had the greatest effect on circulating blood cells, and anemia and neutropenia were most prominent after three series of five daily doses with rest periods between series. ICRF-187 was more toxic to dogs in terms of total dose received when administered in five daily doses than in single doses. A rest period between series of five daily doses allowed a larger total dose to be administered.

[Cancer Treat Rep 64:1211–1215, 1980]

A number of bisdioxopiperazines have been shown to have potent antitumor activity. Significant growth inhibition of Sarcoma 180 or increases in survival time of mice inoculated with L1210 leukemia occurred after treatment with these compounds (1). Growth of other mouse tumors, including Ca 755 and the Lewis lung tumor, can be inhibited by these compounds to varying degrees. These are potent antimetastatic agents that inhibit the development of metastases from primary implants of the syngeneic Lewis lung carcinoma in C578L mice (2). The bisdioxopiperazines have a modest antimitotic activity, but because this activity is not selective, normal cells as well as malignant replicating cells are inhibited. These agents block the cell generation cycle in the late G_2 or possibly G_2/M phase (3). The mechanism of action of the bisdioxopiperazines is not known (4). They are closely related to EDTA and are able to act as in vitro and in vivo chelating agents, but so are closely related compounds that are devoid of antitumor activity. In man, the poor selectivity of the compounds results in poor performance when a compound is used alone (5). Most bisdioxopiperazines, however, potentiate most of the currently available antitumor compounds while simultaneously reducing the toxicity of other anticancer agents such as daunorubicin (5,6).

Previous studies have evaluated the oral toxicity of the bisdioxopiperazine ICRF-159 in rhesus monkeys and beagle dogs (7). Dose-dependent bone marrow and intestinal toxic effects were seen in both species. ICRF-187, the *d*-isomer of the racemic mixture ICRF-159, is more soluble in aqueous fluids than the racemate (8). This study was conducted to determine the toxic effects of ICRF-187 in dogs when it is administered by iv infusion in single- and multiple-dose regimens.

MATERIALS AND METHODS

Experiments were performed on beagle dogs in accordance with the procedures outlined by Prieur et al (9) and modified by the Battelle Columbus

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Laboratories Toxicology Program Office.[1] Dogs were supplied by the Animal Genetics and Production Branch of the National Cancer Institute, through the Battelle Toxicology Program Office. Dogs were housed in individual steel cages, with water available ad libitum. Prior to being selected for study, the dogs had fecal examinations for parasites. Preclinical toxicologic evaluation of ICRF-187 in dogs included physical and ophthalmic examinations, hematologic and serum chemical determinations, urinalyses, necropsies, and histopathologic evaluations. At least two complete hematologic and serum chemical determinations were performed on each animal before treatment. Dogs were treated in pairs, one male and one female at each dose level. Animals were observed up to 45 days after treatment.

The chemical identity of ICRF-187 was verified by infrared spectroscopy, melting point, and optical rotation measurements. The solubility in nonpyrogenic sterile 0.9% sodium chloride was > 10 mg/ml at room temperature (21°C–23°C). This concentration was used for in vitro blood compatibility and protocol studies. Drug solutions were prepared immediately before iv treatment.

Because of the large volume of drug solution (up to 2000 ml/day), all drug doses and the vehicle were administered by iv infusion at rates of 3.8–4.9 ml/minute. Constant infusion rates were maintained with a continuous infusion pump. The saphenous vein was cannulated with a sterile, nonpyrogenic catheter connected to sterile pump tubing. The dogs were restrained in slings during the infusion.

The following definitions of dose levels were used in this study:

Highest nontoxic dose (HNTD)—the highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose produces the aforementioned alterations.

Toxic dose low (TDL)—the lowest dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces no lethality.

Toxic dose high (TDH)—the dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality.

Lethal dose (LD)—the lowest dose which produces drug-induced death in any animal during the treatment or observation period.

[1]Procedures Manual, Battelle Columbus Laboratories, Feb 5, 1975.

RESULTS

A 10-mg/ml solution of ICRF-187 was added to various quantities of canine serum, plasma, or whole blood. Final drug concentrations of up to 5 mg/ml were compatible in these biologic fluids. The defined toxicity levels in dogs are given in table 1.

Single-Dose Study in Dogs

Four pairs of dogs were infused iv with single doses of 2000, 1000, 500, or 250 mg/kg (40,000, 20,000, 10,000, or 5000 mg/m^2). The male treated with 2000 mg/kg was found moribund 3 days after treatment, while the female survived to the end of the observation period. Clinical signs of toxicity during and after treatment were emesis, diarrhea, anorexia, lethargy, weight loss, and hypothermia. The female showed recovery from toxic effects on Day 5. Reduction in food consumption and weight loss were the clinical signs of toxicity at 1000, 500, and 250 mg/kg.

Changes in hematologic and serum chemistry values on Day 2 for both dogs treated with 2000 mg/kg were neutrocytosis, lymphopenia, and increased values for BSP retention, SGOT, SGPT, and prothrombin time. Blood collected from the moribund dog on Day 4 showed hemoconcentration and markedly elevated values for BUN, creatinine, SGOT, SGPT, total and direct bilirubin, and prothrombin time. A urine sample from this dog was acidic, cloudy, and brown-colored, and contained rbcs and wbcs. On Day 4, the female had anemia, leukopenia, hypoglycemia, proteinuria, and increased values for liver function tests. Serum indices of hepatic dysfunction in this dog remained elevated throughout most of the 45-day observation time. Decreased rbc and wbc counts, increased SGOT levels, and proteinuria were observed in both dogs at a dose of 1000 mg/kg. The male also showed a marked increase in BSP retention and SGPT values. Moderate neutrocytosis and lymphopenia occurred at 500 mg/kg with a delayed increase in urinary protein in the dog held for observation. At the lowest dose administered, very slight increases in SGOT values were observed. The male had a slight reduction in wbc count, and the female showed reductions in rbc count, hematocrit, and hemoglobin values.

Microscopic lesions were less severe in the dogs held for 45 days at all doses. At the LD, the dog that died had generalized hemorrhage of tissues; necrosis of lymphoid tissue, small intestinal mucosa, and renal proximal convoluted tubules; cytoplasmic vacuolation in hepatocytes, pancreatic acinar cells, and lining cells of proximal convoluted tubules; and

TABLE 1.—Defined* toxicity levels of ICRF-187 in dogs

Study	Toxicity	Daily dose	
		mg/kg	mg/m²
Single dose	LD	2000.0	40,000.0
	TDH	1000.0	20,000.0
	TDL	500.0	10,000.0
	HNTD	250.0	5000.0
Five daily doses	LD	250.0	5000.0
	TDH	125.0	2500.0
	TDL	31.3	625.0
	HNTD	15.6	312.5
Five daily doses, repeated two times	TDH	125.0	2500.0
	TDL	31.3	625.0
	HNTD	15.6	312.5

**From* ref 9.

bone marrow hypoplasia with an increased myeloid:erythroid ratio. Histopathologic changes 45 days after treatment with this dose were hepatocellular cytoplasmic vacuolation and focal necrosis, hyperplasia, hemorrhage, histiocytosis, erythrophagocytosis, and hemosiderosis in lymphoid tissue. Only the dogs necropsied on Day 8 had necrosis of lymphoid tissue and the lining cells of the proximal convoluted tubules; mild focal necrosis of lymphoid tissue was observed.

Five Daily Dose Study in Dogs

Seven pairs of dogs were studied. One pair was a vehicle control (25 ml/kg), and six pairs were treated once daily for 5 consecutive days at the following levels: 500, 250, 125, 62.5, 31.3, or 15.6 mg/kg/day (10,000, 5000, 2500, 1250, 625, or 312.5 mg/m²/day). Dogs treated with 500 mg/kg died after the fourth or fifth dose. At doses of 250 mg/kg, the male was necropsied according to protocol on Day 6, while the female died on Day 8. Clinical signs of toxicity after both doses were anorexia, emesis, hematemesis, weight loss, and hematochezia. The male treated with the highest dose level was hypothermic 3 hours after the end of each day's treatment. On the last day of dosing, his rectal temperature of 98.8°F before infusion decreased 0.8°F over a 4-hour infusion period. Normal rectal temperatures for beagle dogs in this laboratory are 100°F–102°F. Reduced food consumption and weight loss occurred after doses of 125, 62.5, and 31.3 mg/kg. Reduced food intake during the 5 days of treatment was observed after infusions of either 15.6 mg/kg or the vehicle. No other signs of toxicity were seen in the vehicle control animals.

Changes in blood values at 500-mg/kg doses were decreases in rbc count and alkaline phosphatase (AP), leukopenia, and increased SGOT and SGPT. The female also had increased BUN and creatinine values. At 250 mg/kg, leukopenia, thrombocytopenia, increased BSP retention, increased SGOT and SGPT levels, and mild reductions in potassium and calcium values were observed. At 125 mg/kg, reductions in wbc and platelet counts, decrease in percent neutrophils, and increases in SGOT and SGPT levels occurred. Quantitative changes in some liver function tests (SGPT values, BSP retention, and total and direct bilirubin) were greater at 62.5 than at 125 mg/kg. The effects of 62.5 mg/kg were leukopenia, a differential shift, increased SGOT, and slight reduction in rbc count. Lower doses (31.3 and 15.6 mg/kg) produced slight-to-moderate changes in circulating blood cells and levels of AP, SGOT, and SGPT.

Dogs treated with either 500 or 250 mg/kg had hemorrhages in lymphoid tissue, intestinal tract, adrenal cortex, and urinary bladder; necrosis in lymphoid tissue and mucosa of intestinal tract; hepatocellular cytoplasmic vacuolation; and bone marrow hypoplasia. At the 125-mg/kg level, histologic lesions in the dog necropsied on Day 6 showed lymphoid tissue necrosis, degeneration of proximal convoluted renal tubular cells, petechial hemorrhage in colonic mucosa, cytoplasmic vacuolation of hepatocytes, and bone marrow hypoplasia with an increase in myeloid:erythroid ratio. Microscopic lesions in the dog killed 45 days after treatment with this dose were focal hemorrhage, mild necrosis, histiocytosis, and erythrophagocytosis in lymphoid tissue and swollen hepatocytes. At lower doses, histologic lesions were observed only in the animal killed on Day 6. At 62.5 mg/kg, these lesions included pathologic changes in lymphoid tissue and petechial hemorrhage in the colonic mucosa. Similar changes were apparent at 31.3 mg/kg as well as

necrosis in the cecal mucosa; cellular necrosis, degeneration, and casts in the proximal convoluted tubules; and cellular necrosis in the collecting tubules. There were microscopic lesions in lymphoid tissue at 15.6 mg/kg.

Multiple-Dose Study With Rest Periods in Dogs

Three pairs of dogs were used in this study. The treatment schedule was five consecutive daily infusions for three treatment periods with intervening 9-day rest periods. Dose levels used were the defined TDH, TDL, and HNTD from the five daily dose study in dogs: 125, 31.3, or 15.6 mg/kg (2500, 625, or 312.5 mg/m^2). Clinical signs of toxicity at the 125-mg/kg level were reduced food consumption and weight loss during the three treatment periods. The female showed a greater reduction in food intake than the male and also had a reduced food consumption during the recovery periods. The female of the pair treated with 31.3 mg/kg also showed reduced food intake during and after treatment and weight loss during the first two series. The male had slight reductions in food intake on 4 days during the 5 weeks of the study. At 15.6 mg/kg, only the female reduced her food intake and lost body weight.

At the highest dose level, changes in serum chemistry values were observed during the treatment periods, whereas maximum reduction in circulating blood cells occurred after the end of dosing. SGOT and SGPT levels showed the greatest increases during the first series of treatments. The female also had a moderate increase in AP and a marked increase in BSP retention. Depressions of wbc and platelet counts were most prominent after the first treatment period; a decrease in percent neutrophils occurred after each series. The maximum decrease in rbc count occurred at the end of the third series. Neutropenia, anemia, and slight increases in SGOT and SGPT were observed at 31.3 mg/kg. Mild decreases in rbc and wbc counts and very slight increases in SGOT were present after doses of 15.6 mg/kg.

Microscopic lesions were not present in dogs necropsied 45 days after treatment at any dose. Histopathology was seen in dogs necropsied 24 hours after the last dose. Swollen, vacuolated hepatocytes and an increased bone marrow myeloid:erythroid ratio were observed in the dogs treated with the highest dose level. At 31.3 mg/kg, the dog had focal hemorrhage, erythrophagocytosis and histiocytosis in the lymph nodes, and an increased bone marrow myeloid:erythroid ratio. Histopathologic changes in lymph nodes of the dog treated with a dose of 15.6 mg/kg were similar to those at 31.3 mg/kg.

DISCUSSION

ICRF-159, a bisdioxopiperazine and derivative of EDTA, has been shown to be active against experimental tumors in a variety of model systems (10,11). The toxicity of this agent has previously been determined following oral administration to dogs and monkeys (7). One drawback to the clinical use of ICRF-159 is its poor aqueous solubility (8). ICRF-187, the *d*-isomer of ICRF-159, the racemic mixture, is much more soluble in aqueous fluids and has been previously shown to also process antitumor activity (1). This study was conducted to assess the toxicity of ICRF-187 in dogs.

Toxic effects of ICRF-187 were most prominent on mitotically active tissue: bone marrow, lymphoid tissue, and gastrointestinal mucosa. Similar results were previously seen after ICRF-159 treatment (7). Hepatic and renal toxicity, however, were also evident after iv infusion of ICRF-187. These organ systems were not affected by ICRF-159 (7). Since 50% of ICRF-159 is ICRF-187, the lack of hepatic and renal toxicity seen after ICRF-159 administration may be a function of the total dose of ICRF-187 received. In addition, the present study was performed using the iv route, whereas the previous ICRF-159 study used the oral route. Therefore, the first pass of ICRF-187 (*d*-isomer) through the liver after oral administration of ICRF-159 may be important; it may reduce serum levels, whereas the *l*-isomer may be metabolized to a lesser extent and therefore may be primarily responsible for the observed toxic effects.

Hemorrhage in organs and hemoconcentration were observed after lethal doses of ICRF-187. Bone marrow toxicity was demonstrated by an early neutrocytosis and delayed neutropenia, reduction in rbc and platelet counts, and microscopic evidence of hypoplasia and increased myeloid:erythroid ratios. Three series of repeated doses had the greatest effect on erythrocyte and neutrophil counts. Lymphocytes were also affected by the drug, as shown by a reduction in wbc counts and by microscopic evidence of lymphoid tissue necrosis. Gastrointestinal toxicity was shown by emesis, hematemesis, bloody diarrhea, anorexia, and necrosis of the intestinal mucosa. Toxic effects on the intestinal tract were most prominent at LDs; they were reduced or absent at lower doses.

Marked hepatotoxicity occurred after total doses of 2000–2500 mg/kg on the single-dose or five daily dose schedule. The changes in serum liver function tests were not as severe with a cumulative dose of 1875 mg/kg given in 15 infusions with rest periods between each series of five doses. Serum indices of hepatic function that showed increased values were

SGOT, SGPT, BSP retention, prothrombin time, and bilirubin. In the animal that survived a single infusion of 2000 mg/kg, liver function tests remained elevated above baseline values throughout the 45-day observation period, indicating a persistent effect on liver function. Microscopic evidence of liver damage was the presence of swollen hepatocytes and cytoplasmic vacuolation. The manifestations of hepatotoxicity below LDs were much less severe and had a variable expression. Nephrotoxicity was characterized by changes in serum BUN and creatinine values, which occurred at LDs. Histopathologic lesions in the kidney (necrosis and degeneration of proximal tubule lining cells) were observed at the LD and TDH in the single-dose study and the TDH in the five daily dose study. At lower doses, there was no clinical chemical or histologic evidence of renal toxicity. Although ICRF-187 is a chelating agent, serum calcium, potassium, and sodium concentrations were not altered following treatment.

Schedule-dependent toxicity of ICRF-187 in dogs was apparent in the larger doses which could be administered as a single dose versus five daily infusions. With a single infusion of 2000 mg/kg, one animal died, while the other dog survived treatment. When a total dose of 1250 mg/kg was given over five daily infusions, one dog died. The defined doses (TDH, TDL, and HNTD) for the repeated dose schedule were also lower than for single infusions. Rest periods between series of five daily doses, however, allowed a total dose of 1875 mg/kg to be given without lethality. Thus, larger total doses can be given as a single infusion or on a schedule in which smaller daily doses are administered with rest periods between series.

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Radionuclide Angiography to Predict Patient Response to Hepatic Artery Chemotherapy[1]

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SUMMARY

Direct hepatic artery infusions of chemotherapeutic agents have been used for the treatment of hepatic metastases with some evidence of an increase in survival. Reports of response rates have, in all probability, not accurately estimated the relationship between antitumor drug effect and actual sites of drug distribution within the liver. We have utilized a method of infusing radionuclides at flow rates precisely equal to those used for drug delivery to define patterns of intrahepatic perfusion. This technique has allowed the prediction of patient response to intra-arterial chemotherapy. Nineteen patients with proven hepatic tumors were studied. Baseline technetium-99m (^{99m}Tc)-sulfur colloid (SC) liver-spleen scans were obtained. Hepatic artery catheters were then placed and radiotracer patterns of intrahepatic perfusion were assessed. This was done by infusing 4.0 millicuries (0.2 ml vol) of ^{99m}Tc-macroaggregated albumin via an automated pump at two flow rates: those employed for drug therapy (10–21 ml/hour) and those used for administration by hand injection (1.0 ml/second). Based upon the presence or absence of ^{99m}Tc-macroaggregated albumin flow to focal tumor defects or tumor-involved hepatic lobes as defined on prior ^{99m}Tc-SC liver scans, predictive statements regarding drug response could be made. Response was defined as a ≥ 1.0-cm decrease in either focal defect or size of a hepatic lobe as measured on followup ^{99m}Tc-SC liver scan. Ten of 11 patients evidencing good radionuclide flow to tumor areas showed response; in two cases, radioaggregates were seen within tumor-bearing areas only at high (1.0 ml/second) flow rates. None of eight patients with poor or absent tumor flow showed response. This technique appears to have utility for directing initial catheter placement, sequential followup to monitor patterns of drug distribution, and defining the appropriate flow rate to deliver maximal drug to tumor-bearing regions of the liver.

[Cancer Treat Rep 64:1217–1222, 1980]

The presence of hepatic metastases carries a grave prognosis. The median survival of 390 patients with liver metastases was 75 days, with only 7% of patients alive after 1 year (1). However, direct hepatic artery infusions of chemotherapeutic agents appear to produce an improved response rate manifested by some increase in survival (2,3). In patients in whom systemic treatment with the same drug had been previously unsuccessful, hepatic artery infusions have been shown to produce a four-fold increase in tumor exposure when compared to

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TABLE 1.—Summary of patient data

Patient No.	Tumor	Drug	No. of treatment days
1	Ampulla	Dichloro-methotrexate	9
2	Adenocarcinoma	5-FUdR	42
3	Cholangiocarcinoma	Doxorubicin	3
4	Lung	5-FUdR	35
5	Breast	5-FUdR	7
6	Breast	5-FUdR	16
		Doxorubicin	3
7	Breast	Doxorubicin	6
8	Breast	Doxorubicin	21
9	Colon	5-FUdR	30
10	Colon	5-FUdR	27
11	Colon	5-FUdR	21
12	Colon	5-FUdR	38
13	Colon	5-FUdR	48
14	Colon	5-FUdR	41
15	Colon	5-FUdR	37
16	Colon	5-FUdR	49
17	Colon	5-FUdR	21
18	Hepatoma	Neocarzinostatin	5
19	Hepatoma	5-FUdR	37

levels achieved by peripheral iv administration; using this mode of therapy, regression of liver metastases has been documented (4,5).

The improved selectivity of hepatic drug infusion requires that the chemotherapeutic agent be injected into the afferent blood supply of the tumor, allowing drug extraction during the "first pass" of blood through the liver. A technique capable of accurately defining first-pass intra-arterial patterns of drug flow might allow identification of those patients who, based on perfusion patterns, would be most likely to benefit from infusion chemotherapy. We have recently shown that "slow-flow" intra-arterial infusion of technetium-99m (^{99m}Tc)-sulfur colloid (SC) was a better indicator of hepatic drug distribution than was contrast angiography (6), and suggested the potential advantages of utilizing ^{99m}Tc-macroaggregated albumin (MAA) as a radiotracer. Our observations supported those of others (7–11) which indicated that deposition of radioaggregates reflects first-pass capillary blockade rather than phagocytosis. In the current study, based on the scintigraphic distribution patterns of ^{99m}Tc-MAA introduced into the liver during hepatic artery infusion, we attempted to predict the response of liver tumors to subsequent intra-arterial chemotherapy administration.

MATERIALS AND METHODS

Nine male and ten female patients ranging in age from 25 to 65 years with biopsy-proven primary or metastatic hepatic tumors were evaluated (table 1). Hepatic artery catheters were placed either by the percutaneous route via the axillary or femoral arteries or intra-operatively into the common hepatic artery. Baseline liver-spleen scintiphotographs were obtained on all patients within 1–49 days (mean, 17) prior to the initiation of intra-arterial therapy. Imaging was begun approximately 15 minutes following iv administration of between 3.0 and 4.0 millicuries of ^{99m}Tc-SC.[4] Anterior and posterior scintiphotographs representing 630,000 counts and right and left lateral images of 540,000 counts were collected on a large field-of-view gamma scintillation camera[5] using a low-energy all-purpose parallel-hole collimator. For each patient, an additional anterior view was obtained with a 1 × 10-cm lead strip positioned with the long axis over the right costal margin at the midclavicular line. This marker served as both a standard for measuring the size of the liver and the intrahepatic metastases as well as

[4]New England Nuclear Corp, North Billerica, MA.

[5]Siemens Gammasonics, Des Plaines, IL (FWHM 5.2-mm intrinsic resolution).

providing an anatomic reference for patient positioning during subsequent radionuclide infusion studies.

Intra-arterial infusions of ^{99m}Tc-MAA were performed within 48 hours of catheter placement. Patients were placed in a supine position under the gamma camera, allowing anterior views of the entire abdomen to be obtained. Again, a large field-of-view camera with a low-energy all-purpose parallel-hole collimator was used.

Approximately 4.0 millicuries of the radiopharmaceutic in a volume of < 0.2 ml was introduced into the arterial line via a three-way stopcock. A portable infusion pump[6] was immediately attached to the distal end of the catheter; an initial pump setting of 121 ml/hour was used to carry the injectate into the field of view. As radiotracer entered the distal aspect of the catheter, flow rate was reduced to 10–21 ml/hour (2.7×10^{-3} to 5.8×10^{-3} ml/second) and the radioaggregates were infused into the liver at the precise rate utilized for chemotherapy administration.

Image recording was begun with appearance on the camera persistence scope of radioactivity in the distal catheter. Image collection was performed on a multi-imaging device[5] at time intervals of two frames per second for a total of 80 frames. Following the initial first-pass deposition of ^{99m}Tc-MAA within the liver, a 500,000-count anterior image was obtained.

Immediately following the completion of the pump infusion, a rapid hand flush (approximately 1.0 ml/second) of 10 ml of saline via the three-way stopcock was performed. Residual radioaggregates adherent to the lumen of the catheter frequently provided sufficient radioactivity for this rapid-flush study. If, however, no residual tracer remained, an additional 3–5 millicuries of ^{99m}Tc-MAA was introduced via the stopcock prior to bolus administration. Imaging and recording of this rapid-flow data were done in an identical fashion to that used to obtain the slow-flow information.

Prediction of response to chemotherapy was based on the pattern of distribution of radiolabeled macroaggregates when compared with the location of the focal defects identified on the baseline ^{99m}Tc-SC liver-spleen studies. When the radionuclide infusion data indicated distribution of tracer within the tumor site, a response to chemotherapy was predicted. If postinfusion images indicated absent perfusion to a tumor-bearing area of the liver or extrahepatic activity (ie, extravasation or gastric, renal, or splenic uptake), a prediction of no chemotherapeutic response was made.

[6]Model 922, Imed Corporation, San Diego, CA.

The effects of chemotherapy were based on interpretation of followup ^{99m}Tc-SC liver images. Response to treatment was equated with a $\geq$ 1-cm decrease in liver size, measured as both the maximal, vertical, and horizontal span of the right or left hepatic lobes on the anterior view, and/or a $\geq$ 1-cm decrease in size of perfused focal defects, measured as the maximal height and width of the defect identified on the anterior view. Only focal abnormalities with a diameter of $\geq$ 3 cm which evidenced well-demarcated margins and were localized to high-contrast areas of the liver were selected for evaluation. A fall in the serum alpha-fetoprotein (AFP) was utilized as an indicator of response in one patient with hepatoma in whom followup ^{99m}Tc-SC liver-spleen studies could not be obtained. All ^{99m}Tc-SC liver-spleen studies were performed within 1–42 days (mean, 23) of ^{99m}Tc-MAA infusion examinations.

RESULTS

Based on the criteria of presence or absence of ^{99m}Tc-MAA within focal hepatic defects or the degree of perfusion to diffusely tumor-involved hepatic lobes, the response to intra-arterial chemotherapy was correctly predicted in 18 of 19 patients (fig 1, table 2).

Eleven patients evidenced radioaggregate perfusion to either focal defects or to liver diffusely involved with tumor. In two of the 11 patients, areas of poorly perfused liver at flow rates of 5.8×10^{-3} ml/second showed improved perfusion when radioaggregates were administered at 1.0 ml/second (fig 2). The positive responses seen in ten of the 11 patients were manifested as 13 instances of a decrease in size of the right and/or left tumor-involved hepatic lobes and five instances of a decrease in size of discrete focal defects. One patient demonstrated good tracer perfusion to tumor yet showed no change on followup ^{99m}Tc-SC scan.

Of the eight patients who showed no tumor perfusion, one demonstrated an increase in focal defect size and seven showed no tumor regression when comparing pre- and post-chemotherapy liver-spleen images.

Patient 19 had no followup ^{99m}Tc-SC images performed; however, response was assessed by sequential monitoring of AFP levels which showed a steady decline from 12,000 to 6000 ng/ml over the 37 days of chemotherapy.

DISCUSSION

Intra-arterial infusions of radio-iodinated macroaggregates of albumin (7–11) and ^{99m}Tc labeled to

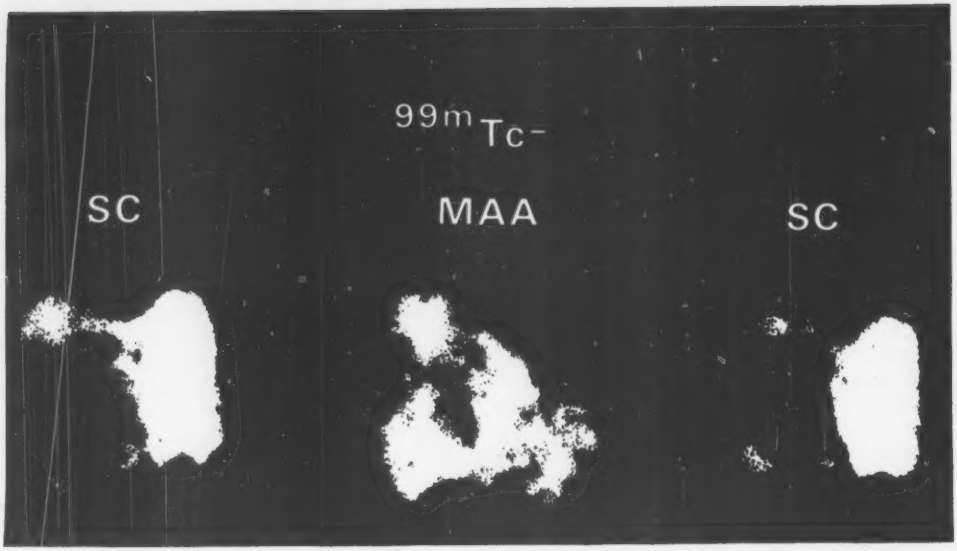

FIGURE 1.—^{99m}Tc-SC liver scan (left) shows large focal defect in right hepatic lobe. Perfusion study (center) indicates adequate flow to entire liver. Followup radiocolloid liver scan (right) shows marked regression in hepatic size (patient 2).

TABLE 2.—Correlation between ^{99m}Tc-MAA perfusion* and response to chemotherapy as manifested on ^{99m}Tc-SC liver-spleen images in 19 patients

Perfusion		Hepatic lobe/focal defect size	
Yes	No	Decrease	Increase
10†	—	10	—
1	—	—	1
—	8	—	8

*Perfusion of either a hepatic lobe diffusely involved with tumor or a focal defect.

†Values = No. of patients.

microspheres, MAA, and gelfoam (12–14) have been reported. However, none of these previous studies has focused upon the information available from intra-arterial radionuclide distribution patterns obtained when infusions are performed at varying flow rates. Also, none of these previous reports has addressed the relationship between radionuclide perfusion patterns and changes in the intra-hepatic tumor following therapy.

Use of ^{99m}Tc-MAA with a mean particle size of approximately 25,000 nm distributes independently of reticuloendothelial cell function localizing within the liver by capillary blockade. This method of localization thus circumvents the problems of recirculating tracer as seen when ^{99m}Tc-SC is employed. Use of ^{99m}Tc-MAA provides an accurate depiction of first-pass drug distribution, and since the deposition of these radioaggregates is cumulative, their intrahepatic distribution at flow rates of 2.7–5.8 $\times 10^{-3}$ ml/second can be defined.

Ionic ^{99m}Tc as pertechnetate ($^{99m}TcO_4^-$) infused at a slow-flow rate provides initial first-pass hepatic images identical to those obtained with ^{99m}Tc-MAA (fig 3). This suggests that the patterns of macroaggregate distribution accurately reflect arterial infusions of soluble drugs.

For patients receiving either percutaneous or surgically placed catheters, information regarding slow-flow perfusion can be of extreme clinical importance. High flow rate contrast studies at the

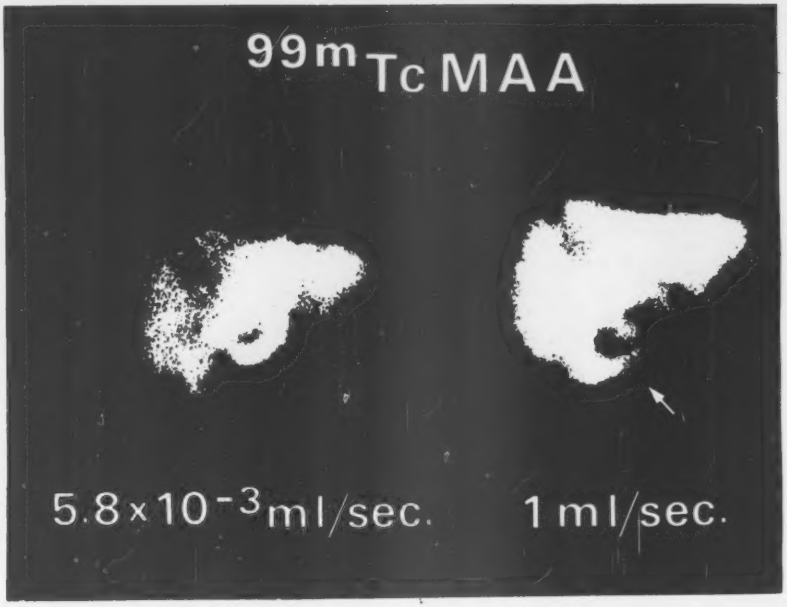

FIGURE 2.—At flow rate employed for infusion therapy (left) there is inadequate perfusion of focal defect at inferior margin of liver. Higher flow rate indicates good perfusion of this lesion (arrow) (patient 7).

time of catheter placement may demonstrate that the catheter tip, positioned in the common hepatic artery just beyond the origin of the gastroduodenal artery, adequately and exclusively perfuses the right and left hepatic lobes. It is only with the slow-flow radionuclide study that retrograde perfusion of the gastroduodenal artery may be demonstrated.

Visualization of extrahepatic activity carries important clinical implications. Radioaggregate collection within vessels such as the gastroduodenal artery has been associated with potential gastrointestinal and systemic toxicity, since the degree of chemotherapeutic agent detoxification within the liver becomes diminished. A pattern of extrahepatic flow has also been highly predictive of an absence of tumor response following chemotherapy. In two of our eight nonresponding patients, collections of extrahepatic radioaggregates were noted.

From our more recent experience, it now appears possible to precisely tailor the flow rate for each individual to assure optimal hepatic tumor perfusion (fig 2). This would allow optimal drug delivery and potentially a maximal therapeutic response. Two additional patients not included in this study who demonstrated perfusion of their hepatic tumors only at the higher flow rates have since been treated by periodically administering intra-arterial drugs at 1 ml/second throughout the day. Both patients, previously nonresponders using the ^{99m}Tc-SC scan as the criterion, showed a decrease in tumor size on followup liver imaging.

Although we have not addressed the issue of tumor response based on individual chemotherapeutic agents used, this technique should allow us to better assess therapeutic results. Reported response rates in most hepatic arterial therapeutic studies, to date, have probably been unable to estimate the true antitumor drug activity as related to actual sites of drug delivery into tumor-bearing areas of the liver. This radionuclide technique that we have described will allow us to differentiate between those patients in whom a nonresponse may be due to a lack of adequate drug perfusion and those in whom a nonresponse is due to a failure of drug effect. For instance, patient 18 demonstrated an excellent tumor blush at a flow rate of 5.8×10^{-3} ml/second. A failure to respond to chemotherapy could not be explained on a technical basis; rather, the neocarzinostatin may simply have been ineffective in this clinical situation.

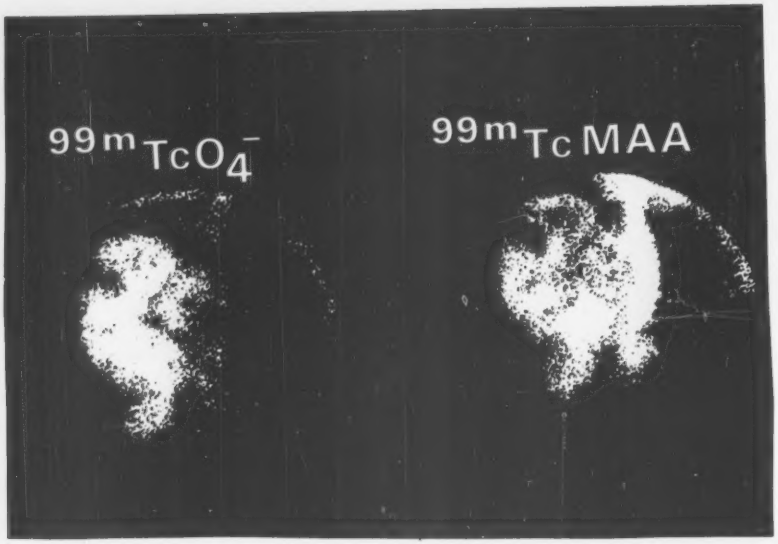

FIGURE 3.—Infusion of $^{99m}TcO_4^-$ and ^{99m}Tc-MAA at 5.3×10^{-3} ml/sec is seen to produce identical patterns of intrahepatic distribution (patient 10).

Radionuclide infusions, which enable the clinician to define the intrahepatic distribution of blood flow (the critical factor responsible for patient response to intra-arterial drug therapy), provide a pictorial representation of changes in catheter position and the subsequent alterations in perfusion at the precise flow rates utilized for drug administration. Correlation of flow distribution patterns with both clinical course and chemotherapeutic agents employed will establish the potential role of this technique in patients undergoing intra-arterial drug infusion therapy.

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Renal Handling of *cis*-Diamminedichloroplatinum(II)[1,2]

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SUMMARY

The renal handling of *cis*-diamminedichloroplatinum(II) (cisplatin) was investigated by measuring the renal clearance of creatinine and free platinum (Pt). Free Pt clearance exceeded the glomerular filtration rate, indicating secretion of cisplatin or a metabolite. These results suggest that accumulation of Pt within renal tubular cells may be related to its nephrotoxicity.

[Cancer Treat Rep 64:1223–1226, 1980]

Cis-diamminedichloroplatinum(II) (cisplatin) is an important chemotherapeutic agent, but its optimal use is limited by nephrotoxicity (1–3), the mechanism of which is unknown (4,5). Although nephrotoxicity has been reduced in man through either mannitol diuresis (6) or administration of the drug by 24-hour infusion (7), diminished renal function may limit long-term use of the drug. Although the major route of platinum (Pt) excretion is via the urine, the mechanism of renal handling of Pt has not been described. To investigate the mechanism of nephrotoxicity, this study was designed to measure renal handling of the drug.

MATERIALS AND METHODS

Six patients with advanced head and neck cancer received cisplatin at 50–80 mg/m² by 24-hour infusion (7). There were five men and one woman, with a mean age of 58. The mean baseline creatinine was 1.2 mg/100 ml (range, 0.8–1.6 mg/100 ml), and mean baseline creatinine clearance was 72 ml/minute (range, 34–128 ml/minute). Two additional patients received cisplatin at 100 mg/m² for 30 minutes, with mannitol diuresis (6).

Multiple timed urine samples were collected during the infusion and in the 24-hour rehydration period. Urine samples were analyzed for creatinine and total Pt. Blood samples were taken before and after the urine collections and were analyzed for creatinine and plasma Pt (total and free). Blood samples were obtained by venipuncture and immediately centrifuged at 0°C, and the plasma was removed. Plasma ultrafiltrates were obtained immediately by centrifugation with Amicon ultrafilters at 0°C. A series of experiments demonstrated that the rate of Pt binding of plasma proteins was slowed markedly at 0°C. Free and total Pt were determined with a Varian 175 flameless atomic absorption spectrophotometer. Free Pt clearances were calculated and compared with simultaneous creatinine clearances.

RESULTS

With delivery by 24-hour infusion, the peak total plasma Pt level occurred at 24–28 hours, with a mean of 1.9–2.0 μg/ml (fig 1). Binding increased with time, and by 12 hours, plasma Pt was > 90% bound. The free Pt concentration was stable between 4 and 24 hours. In every clearance period, the free Pt clearance exceeded the creatinine clearance (fig 2). When all clearance periods were combined, the free Pt clearance was 156% of the creatinine clearance; this was statistically significant ($P < 0.05$). Two patients received cisplatin by iv bolus, with concomitant mannitol diuresis (table 1).

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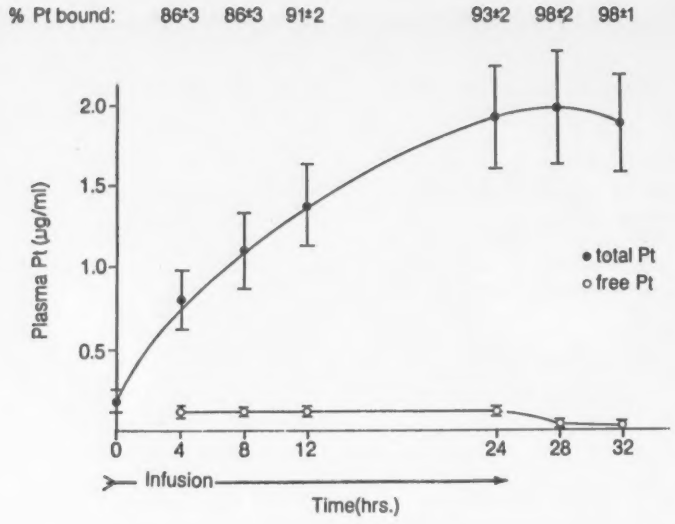

FIGURE 1.—Plasma Pt during and following 24-hr infusion. Data = mean ± SE of total Pt (●) and free Pt (○).

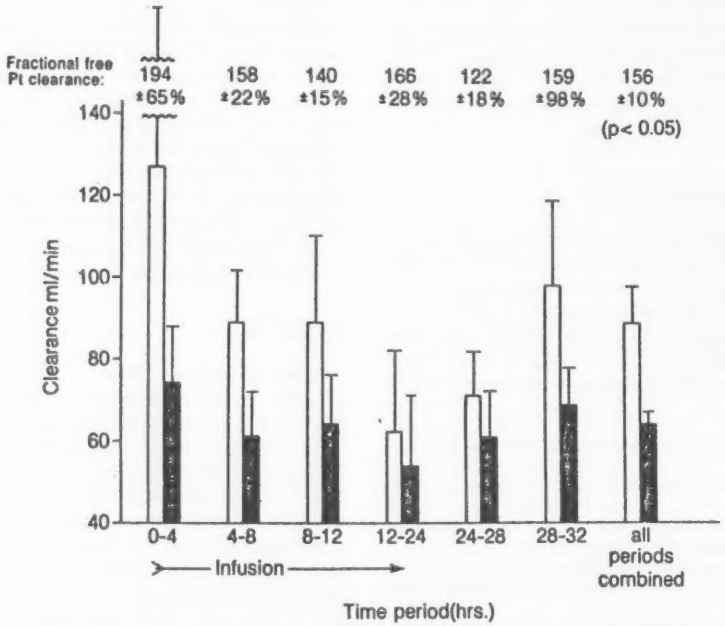

FIGURE 2.—Creatinine and free Pt clearances during and following 24-hr infusion. Fractional free Pt clearance = free Pt clearance/creatinine clearance. Data = mean ± SE of the free Pt clearance, ml/min (open vertical bars) and creatinine clearance, ml/min (filled vertical bars). *P* value determined by paired t test.

TABLE 1.—Fractional Pt clearances following iv bolus of cisplatin

Time (hrs)	Mean free Pt (μg/ml)	Mean Pt clearance (ml/min)	Mean creatinine clearance (ml/min)	Mean fractional Pt clearance (%)*
1–2	0.43	128	75	170
2–3	0.09	658	176	362
3–4	0.08	314	174	192
4–8	0.08	117	114	111

*Expressed as free Pt clearance/creatinine clearance.

There was a rapid decline in Pt following the bolus, and free Pt reached a steady state after 4 hours. The free Pt clearance exceeded the creatinine clearance in the first 8 hours after infusion.

DISCUSSION

The present results show that the renal clearance of free Pt (cisplatin or metabolites) exceeds the glomerular filtration rate, suggesting that, in addition to excretion by filtration, cisplatin or a metabolite is secreted by the human kidney. This suggestion is based on certain assumptions. The first assumption is that the endogenous creatinine clearance is a good estimate of the glomerular filtration rate. Creatinine is filtered and is not reabsorbed by the renal tubule, but some creatinine may be secreted. Creatinine clearance may be a slight overestimate of the glomerular filtration rate. Thus, the fractional Pt clearances may be underestimated, an additional indication of Pt secretion. The second and more important assumption is that the fraction of Pt that is bound to plasma proteins is not overestimated by the present methods. If protein binding continued in vitro prior to measurement, or if the present method overestimates binding, then the concentration of free Pt in plasma would be underestimated. This would increase the calculated Pt clearance. Experiments in this laboratory have demonstrated that protein binding is markedly slowed at 0°C; therefore, these results appear to be valid.

It should be emphasized that our assay for Pt does not distinguish between cisplatin and other Pt compounds. Therefore, it is possible that a metabolite of cisplatin, rather than cisplatin itself, may be the secreted species. Additional studies are being done to identify the nature of the unbound Pt.

A wide variety of organic acids, organic bases (8), and mercurials (9) are secreted by the kidney. It is generally thought that the secretory process involves active accumulation of the secreted substances in the renal cells and passive transport from the cells into the tubule of the lumen (8). The pars recta of the proximal tubule in the outer medulla is the most active site of secretion. Other studies in our laboratory have demonstrated that the pars recta is the portion of the nephron most damaged by cisplatin. Furthermore, Pt concentrations are greatest in the outer medulla, following cisplatin administration (10).

The observation that cisplatin or a metabolite is secreted has important pharmacologic implications. If cellular accumulation is responsible for secretion, then high intracellular accumulation may cause tubular necrosis. Ross and Gayle (11) have reported that probenecid, an inhibitor of secretion, reduced cisplatin nephrotoxicity in rats. These results are consistent with the suggestion that cisplatin is secreted. Agents that block secretion may be effective in preventing nephrotoxicity and also may diminish renal excretion of the drug; therefore, less drug may be administered with the same benefit and less nephrotoxicity. Other investigations of this are in progress to confirm these preliminary observations.

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Pharmacokinetics of Vinblastine-Loaded Platelets Utilized in the Treatment of Platelet-Phagocytizing Tumors[1,2]

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SUMMARY

Vinblastine-loaded platelets (VLP) have been successfully used in the treatment of idiopathic thrombocytopenic purpura. Three patients with platelet-phagocytizing tumors and thrombocytopenia were treated with VLP. Patient 1 has sustained a response for 11 months. Patient 2 was not evaluable for response because of early death. Patient 3 had a brief partial response. Vinblastine levels were measured by radioimmunoassay in the platelet-rich plasma, platelet-poor plasma, and serum. The pharmacokinetic data obtained on the patients suggest that: (*a*) the amount of vinblastine bound to platelets in vivo is a function of the platelet-poor plasma vinblastine level and the platelet count; (*b*) VLP will probably not have any therapeutic advantage, compared with iv vinblastine alone in patients with normal platelet counts; and (*c*) it appears that delivery of vinblastine was tumor-specific, since the bone marrow serum vinblastine level in patient 2, obtained when the marrow was replaced by tumor cells, was 2.5-fold higher than a simultaneous peripheral blood serum vinblastine level after VLP. Additional studies with VLP appear warranted in patients with platelet-phagocytizing tumors resulting in thrombocytopenia.

[Cancer Treat Rep 64:1227–1233, 1980]

Vinblastine, a vinca alkaloid, is an effective antitumor agent with demonstrated activity against lymphomas and testicular carcinoma (1,2). However, it has shown, at best, modest activity against most other solid tumors (1). Vinblastine binds to tubulin, a protein found in microtubules. Its mechanism of chemotherapeutic effect is probably secondary to its interaction with tubulin in the mitotic spindle, which results in mitotic arrest and cell death (3).

Platelets are very rich in microtubules. Incubation of vinblastine with platelets in vitro can result in significant binding of the drug to the platelets. Vinblastine-loaded platelets (VLP) have been used successfully to treat idiopathic thrombocytopenic purpura. It is postulated that the VLP are phagocytized by macrophages of the reticuloendothelial system. These macrophages are consequently destroyed and the thrombocytopenia is alleviated. Therapy with VLP has been effective when iv vinca alkaloids were not successful (4).

The following report presents the initial results obtained by treating platelet phagocytizing tumors with VLP. Three case reports, along with pharmacokinetic data on these patients, are presented.

MATERIALS AND METHODS

VLP were prepared as previously described (4). Approximately 4–8 × 10^{11} platelets in 300–350 ml

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[4]We thank Drs. J. Robert Smith and Arlan Gottlieb for referral of patients.

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of plasma anticoagulated with ACD-B were harvested through plateletpheresis by using a Haemonetics model-30 blood processor (Haemonetics Corp, Natick, MA) (5). Vinblastine was added to the platelet-rich plasma (PRP) to achieve a concentration of 300 μg/ml. The mixture was incubated in the dark for 1 hour at 37°C, with agitation. After incubation, the platelets were concentrated by centrifugation at 1000 × *g* for 20 minutes at room temperature in a PR-6000 blood bank centrifuge (Damon/IEC, Needham Heights, MA). Supernatant plasma was then removed, and the platelets were resuspended in 100 ml of vinblastine-free, group-specific donor plasma. The VLP were infused within 15 minutes through a Fenwell-4C2100 platelet recipient set; 2 ml of this mixture was removed and assayed for vinblastine.

Following the infusion of VLP (Time 0 was the end of the infusion) or following the administration of vinblastine alone by rapid iv injection, plasma and serum samples were collected at 5, 10, 15, 30, 45, 60, 75, 90, 120, 180, 360, 720, and 1440 minutes. PRP, which is wbc-poor (< 15% of the whole blood wbc count), was obtained by centrifugation of EDTA-anticoagulated whole blood at 200 × *g* for 10 minutes at 22°C. Platelet-poor plasma (PPP) was obtained by centrifugation of PRP at 2700 × *g* for 20 minutes at 22°C. Bone marrow aspirates from the posterior iliac crest were obtained 75 minutes after the infusion of VLP. A small part of the aspirate was used to confirm cell morphology. The rest of the sample was allowed to clot, and the serum was obtained.

The serum, PRP, and PPP vinblastine levels were determined by radioimmunoassay as previously described (6). Antiserum was a generous gift of Dr. M. Root, Eli Lilly Research Laboratories. Standard curves were performed in serum, buffer, and PRP, with equivalent results.

Pharmacokinetic curves were generated by using a Wang-2200 computer with curve-fitting models for the sum of two and three exponentials. The curves generated on this limited number of observation points fit best with a two-compartment model, as described by the sum of two exponentials. The formulation and solution of the equations were derived from the pharmacokinetic parameters according to Wagner (7).

CASE REPORTS

Patient 1

Patient 1, a 49-year-old white female, presented with an undifferentiated carcinoma of unknown primary present in the left supraclavicular nodes, retroperitoneum, and bone marrow. Admission laboratory data included: a hematocrit of 17.7%; a hemoglobin of 5.4 g/100 ml; a wbc count of 16,400/μl with a shift to the left on differential; a platelet count of 4000/μl; and a reticulocyte count of 7.8%. Routine chemistry and clotting studies were normal. Radiographic studies failed to reveal a primary site, although a computerized axial tomography (CAT) scan of her abdomen suggested the possibility that the primary site of the tumor was the pancreas. Figure 1 shows several important aspects of the patient's clinical course.

A bone marrow aspirate from the posterior iliac crest revealed a normal-to-increased number of megakaryocytes with clusters of tumor cells that were enguifing platelets, rbcs, and wbcs. Bone marrow biopsy showed major replacement of the normal marrow by tumor cells.

The patient was initially treated with mitomycin C, doxorubicin (Adriamycin), and cyclophosphamide combination chemotherapy, in addition to a trial of prednisone. She required several rbc and platelet transfusions because of repeated episodes of vaginal bleeding and epistaxis. No significant antitumor response was noted, as measured by repeat abdominal CAT scan, physical examination, repeat bone marrow biopsy, or serial platelet counts.

Since the tumor cells in the patient's bone marrow were phagocytizing platelets, a trial of VLP was started. She received her first two courses 1 week apart, and for the next 5 months she received VLP every 2–3 weeks. The patient showed a dramatic response, as evidenced by: (*a*) return of hematologic parameters to normal (fig 1), (*b*) a 25%–50% reduction in the left supraclavicular adenopathy, (*c*) a return to normal of total protein and albumin levels, and (*d*) a change in the patient's performance status from bedridden to normal activity.

Recently, we have demonstrated that VLP probably have no advantage over iv vinblastine alone in patients with normal platelet counts (*see* Results). Therefore, the patient was switched to iv vinblastine (14–18 mg) every 3–4 weeks and she continued in remission for 5 months with this therapy; total response time was 11 months. She developed recurrent disease in the vertebral bodies of the first two cervical vertebrae and microscopic disease in the bone marrow. The tumor is no longer phagocytizing platelets, and the platelet count has remained normal.

Patient 2

Patient 2, a 52-year-old female, developed malignant histiocytosis with phagocytosis of platelets,

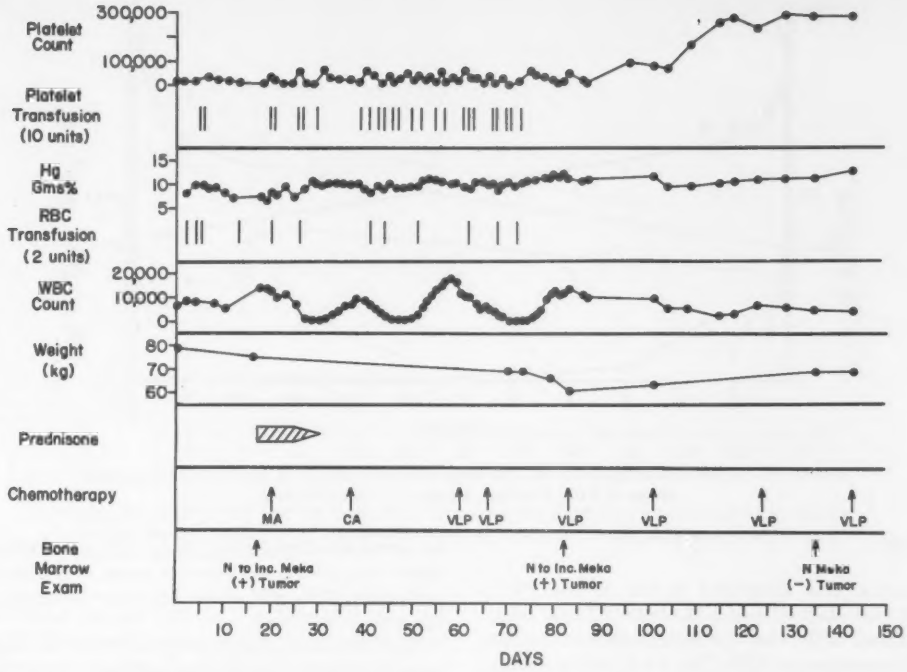

FIGURE 1.—Clinical course of patient 1. M = mitomycin C; A = Adriamycin; C = cyclophosphamide; N = normal; Inc = increased; Meka = megakaryocytes; (+) Tumor = tumor cells in the bone marrow; (–) Tumor = no tumor in the bone marrow.

rbcs, and nuclear debris by tumor cells in the bone marrow. She was treated with cyclophosphamide, doxorubicin, and vincristine chemotherapy. There was progression of the disease, as evidenced by enlarging hepatosplenomegaly. A trial of VLP was started at a time when the platelet count was < 1000/μl, the wbc count was 500/μl, and the total bilirubin was > 20 mg/dl. The patient died of gastrointestinal hemorrhage 3 days after VLP and, therefore, was unevaluable for response. However, a bone marrow aspirate performed 75 minutes after VLP showed (*a*) almost complete replacement by malignant histiocytes and (*b*) a bone marrow serum vinblastine level that was significantly higher than a comparable peripheral blood serum vinblastine level (*see* Results).

Patient 3

Patient 3, a 20-year-old male, presented with undifferentiated carcinoma of the cervical lymph nodes and bone marrow. Routine blood work including clotting studies was normal except for a lactic acid dehydrogenase (LDH) level of 2140 IU/liter. Radiographic studies were done in search of a primary site and revealed a mass in the right maxillary sinus. A biopsy sample of this lesion showed undifferentiated carcinoma. Cytochemical and immunologic cell marker studies indicated that the neoplastic cells were not of lymphocytic or histiocytic origin. Examination of the bone marrow revealed partial infiltration by neoplastic cells phagocytizing rbcs, nucleated cells, and platelets. There were an increased number of megakaryocytes.

The patient's platelet count decreased to 25,000/μl, with normal clotting studies. He was treated twice with VLP. Following the first course of VLP, he sustained a good partial response, as evidenced by a 75% reduction in lymphadenopathy and LDH. The platelet count increased to 100,000/μl. A repeat bone marrow showed a marked decrease in the neoplastic infiltration. Following the second course of VLP, he showed progression of the disease. He was treated with intensive combination chemotherapy including doxorubicin, cyclophosphamide, vincristine, bleomycin, and prednisone. A complete response was achieved and lasted for 5 months.

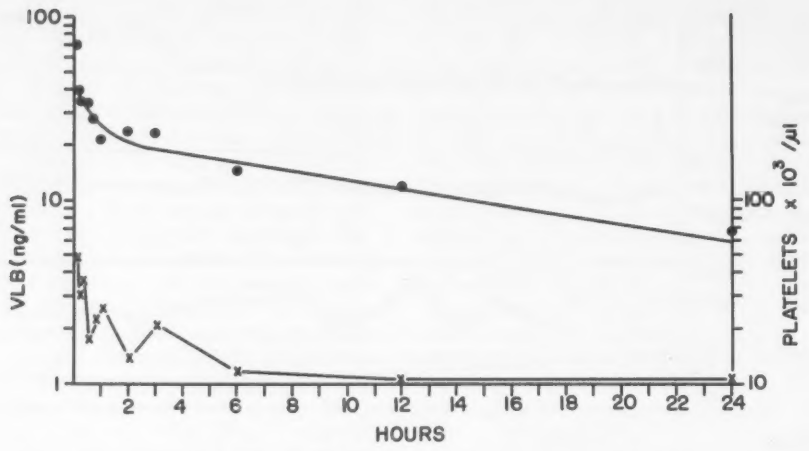

FIGURE 2.—Serum vinblastine levels (●) and serial platelet counts (x) in patient 1 following first course of VLP. Baseline platelet count was 18,000/µl.

RESULTS

Vinblastine was measured in the serum, PPP, and PRP (*see* Materials and Methods). There were no significant differences between measurements obtained on serum or PPP. The VLP packs usually contained 4–10 mg of vinblastine. The percentage of the drug associated with the platelets $\left(\frac{PRP - PPP}{PRP} \times 100\right)$ in the VLP was 10%–30%, ie, 1.7–3.5 µg of vinblastine/10^9 platelets. All of the pharmacokinetic curves generated on the patients' vinblastine levels were best shown by a two-compartment open model. The platelet count is illustrated on the figures only if the baseline platelet count was < 100,000/µl. There was no detectable relationship between the change in the platelet count after VLP and the serum or PPP vinblastine levels (figs 2,3).

Pharmacokinetic curves generated on data from patient 1 are shown in figures 2 and 4. In the pharmacokinetic curve of serum vinblastine levels after the first course of VLP, the β-half-life of the elimination phase was 12.2 hours (fig 2). Unfortunately, a bone marrow serum vinblastine level was not done after the first course of VLP. After the platelet count had returned to 280,000/µl and the bone marrow was free of tumor, the β-half-life decreased to 7.9 hours after VLP. A 75-minute bone marrow serum vinblastine level was the same as a simultaneous peripheral blood serum vinblastine level. The vinblastine levels in PPP and PRP were measured; 60%–80% of the drug was associated with the platelets in vivo after VLP when the baseline platelet count was 280,000/µl (fig 4). After iv vinblastine alone was given, the platelet count was 370,000/µl, and again, 60%–80% of the drug was associated with the platelets (fig 4).

We have confirmed that 60%–80% of the drug was associated with the platelets following iv vinblastine in two other patients with normal platelet counts. Therefore, it appears that patients with a normal platelet count have a similar distribution of drug to PRP and PPP with either VLP or iv vinblastine alone.

The pharmacokinetic curve generated from serum vinblastine levels following the administration of VLP to patient 2 showed a prolonged β-half-life of 89.7 hours, although there was a rapid decrease in the platelet count to baseline within 3 hours. This was not unexpected since the patient was critically ill and had grossly abnormal liver function. The most important finding was that the bone marrow serum vinblastine level at 75 minutes was approximately 2.5-fold greater than the simultaneous peripheral blood serum vinblastine level (78 ng/ml vs 31.5 ng/ml, respectively). The patient's bone marrow was replaced by tumor cells consuming platelets and rbcs at the time of sampling. This result is in marked contrast to the similar bone marrow and peripheral blood serum vinblastine levels after VLP in patient 1, when the marrow was free of tumor. Unfortunately, we do not have serial observations on the same patient.

The pharmacokinetic curve of PRP and PPP vinblastine levels after the first course of VLP in patient 3 is shown in figure 3. These data were obtained when the patient's baseline platelet count

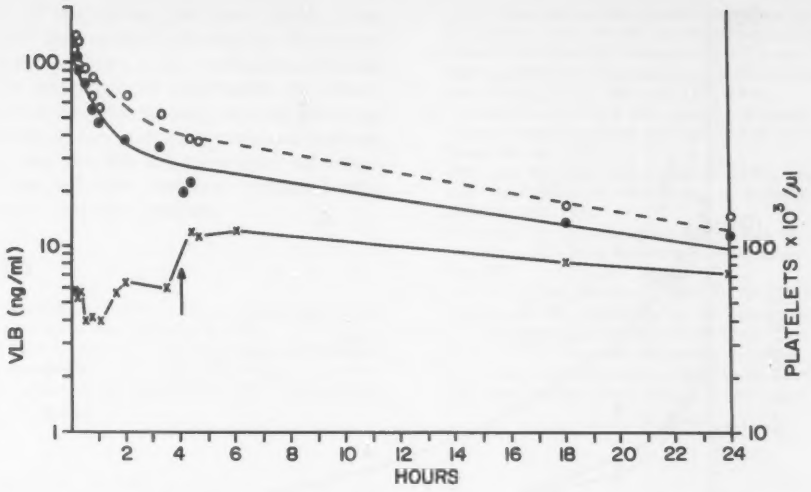

FIGURE 3.—PRP (○) and PPP (●) vinblastine levels of patient 3 following 1st course of VLP. Results of serial platelet counts (x) and transfusion of 10 units of vinblastine-free platelets (arrow). Baseline platelet count was 23,000/µl.

was 23,000/µl. About 25% of the drug was associated with the platelets when the platelet count was approximately 50,000/µl. Four hours after VLP, another 10 units of platelets without any drug was transfused into the patient to increase the platelet count and determine if a greater percentage of the vinblastine would be associated with the platelets. The platelet count was not increased to the extent that had been expected, and there was no increase in the percentage of vinblastine associated with the platelets within 30 minutes. The baseline platelet count prior to the second course of VLP was 73,000/µl. The second course of VLP raised the platelet count to approximately 150,000/µl. Approximately 55% of the vinblastine was associated with the platelets.

DISCUSSION

VLP were initially used to successfully treat idiopathic thrombocytopenic purpura (4). This report suggests that VLP may be useful for malignancies with tumor phagocytosis of platelets, which results in thrombocytopenia. Patient 1 had a good partial response for 11 months. This response occurred in an anaplastic carcinoma in which cyclophosphamide, mitomycin C, and doxorubicin combination chemotherapy failed to produce a response. However, it is not certain that VLP were necessary to produce the original response in patient 1. Perhaps vinblastine alone would have sufficed. Patient 2 died within 3 days of VLP, but there was evidence of selective delivery of vinblastine to the tumor cells in the marrow by VLP. Patient 3 developed a good partial response that unfortunately was of short duration.

The binding of vinblastine to platelets can be demonstrated by radioimmunoassay of PRP and PPP. The vinblastine bound to the platelets is readily exchangeable in vivo since the vinblastine levels in both the PRP and PPP decrease in a similar fashion as a function of time, even when the platelet count remains normal. Previous studies both in vitro and in vivo are in agreement with these results (6,8).

The in vivo ability of the platelets to release vinblastine appears to be similar after VLP or iv vinblastine in patients with normal platelet counts. Not only is there a similar vinblastine release from platelets in these patients, but there is also a similar percentage of drug associated with platelets $\left(\frac{PRP - PPP}{PRP} \times 100\right)$ after iv vinblastine alone or VLP. Approximately 60%–80% of the vinblastine was associated with the platelets following either mode of administration. Therefore, once the platelet count has returned to normal, it is unlikely that VLP will have any therapeutic advantage over iv vinblastine alone. Patient 1 sustained her partial response with iv vinblastine alone for 5 months.

It appears from the data that the percentage of vinblastine associated with platelets in vivo is largely a function of the platelet count and the PPP vinblastine level but is independent of the mode of

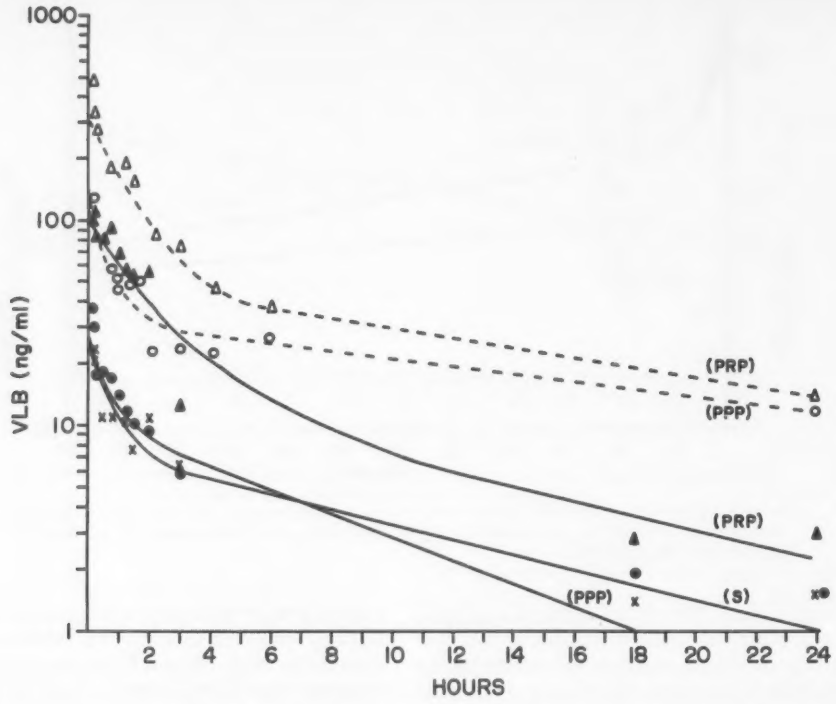

FIGURE 4.—Pharmacokinetic curves generated from PRP, PPP, and serum (S) vinblastine levels of patient 1. Solid lines = curves generated after VLP; x = serum vinblastine levels; ● = PPP vinblastine levels; ▲ = PRP vinblastine levels; interrupted lines = curves generated after iv vinblastine alone; ○ = PPP vinblastine levels; △ = PRP vinblastine levels. Prior to VLP and iv vinblastine alone, the baseline platelet counts were 280,000/μl and 370,000/μl, respectively.

administration (VLP or iv vinblastine). If infused platelets will bind vinblastine as efficiently and rapidly as endogenous platelets, it should be possible to infuse platelets and vinblastine without prior incubation into thrombocytopenic patients and obtain, in vivo, an association of vinblastine to platelets similar to that obtained with VLP. This would eliminate the tedious in vitro incubation process. We plan to treat the next patient who has a platelet-consuming tumor with a mixture of platelets and vinblastine. Appropriate pharmacology studies will be done.

Patient 2 demonstrated a 2.5-fold increase in bone marrow serum vinblastine level, compared with a simultaneous peripheral blood serum vinblastine level following VLP. This increased bone marrow vinblastine level occurred at a time when the bone marrow was replaced by tumor. Patient 1 did not demonstrate a difference in the marrow and peripheral blood serum vinblastine levels after VLP when the bone marrow was free of tumor. These findings strongly suggest specific delivery of vinblastine by VLP to the tumor cells consuming platelets in patient 2. The serum level in the bone marrow may lead to underestimation of the true selectivity of the delivery system since some of the vinblastine in the tumor cells is probably not released in the clotting process (vinblastine in platelets is not released in clotting since the serum vinblastine levels are similar to PPP and not PRP).

The therapeutic index of most anticancer agents is very narrow. There are at least two methods available to change the therapeutic index of anticancer agents. One method is to decrease the toxicity while retaining antitumor activity, eg, leucovorin rescue after methotrexate (9). Another method is to increase the antitumor activity while not changing the toxicity. Selective delivery of antitumor agents is an example of this. VLP may be selective therapy in certain malignancies, ie, tumors that phagocytize platelets, resulting in thrombocytopenia. However, the distribution of VLP to alloimmunized patients is probably altered because of increased uptake of platelets by the reticuloendo-

thelial system of the spleen and liver. After a few courses of VLP, this uptake is probably diminished secondary to cytotoxicity to the reticuloendothelial system (4). In addition, the mechanism of thrombocytopenia in these patients may be a combination of factors including tumor phagocytosis and marrow invasion. Further studies are necessary to determine if VLP are selective therapy compared with vinblastine alone in these patients.

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Cholestatic Jaundice Associated with Chlorozotocin[1]

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SUMMARY

Clinical and pathologic studies of three cases of drug-induced cholestatic jaundice associated with the new antineoplastic agent chlorozotocin are presented. All three patients had received intermittent treatment with high doses of chlorozotocin. The recognition of this unusual form of hepatotoxicity is important in view of the many possible causes of jaundice in cancer patients.

[Cancer Treat Rep 64:1235–1239, 1980]

Hepatotoxicity occurs in up to 26% of patients receiving nitrosoureas (1–8). Although the degree of hepatic dysfunction is usually mild and reversible, occasionally there are severe alterations in liver function and hepatic coma that may contribute to a worsening of the patient's condition (1,2,5,6,8).

Pathologic examinations of the liver have revealed significant hepatocellular necrosis in a few patients (4), while in others the liver has been normal (7). During the recent clinical investigation of chlorozotocin, a new nitrosourea analog, three patients developed elevations in serum enzymes and jaundice with a pathologic picture of intrahepatic cholestasis. The details of these unusual cases are presented.

CASE REPORTS

Patient 1

Patient 1, a 55-year-old male, presented with regional recurrence of a squamous cell carcinoma of the lung. He had previously been treated with a split course of radiotherapy (5000 rads/5 weeks) to the left hilus and mediastinum. Between January and July 1978, he received four courses of chlorozotocin, 225 mg/m² iv, as a single 370-mg total dose at 6-week intervals. In August 1978, his examination revealed scleral icterus and a nontender liver with a 10-cm span. There was no fever or dermatitis. He denied having abdominal pain or pruritis but had noticed darkening of the urine and lightening of the stools. He also denied recent ethanol use or exposure to known hepatotoxins. His medications were ferrous sulfate (300 mg/day), a daily vitamin, and an occasional acetaminophen tablet.

Laboratory evaluation showed a stable hemoglobin concentration; a wbc count of 10,000/μl with no eosinophils; an alkaline phosphatase of 1581 mU/ml (normal, 30–115); an SGOT of 102 mU/ml (normal, 8–40); a GGTP of 347 IU/liter (normal, 9–54); an SGPT of 91 mU/ml (normal, 8–40); and a total bilirubin of 3.5 mg/dl, with a direct fraction of 3.0 mg/dl. The HbSAg was negative, and other laboratory tests were within the normal range.

Liver and spleen scans were normal. Ultrasonographic and computerized tomographic (CT) examinations showed no evidence of dilatation of the intrahepatic or extrahepatic bile ducts; there were no masses, and the gallbladder was normal. Peritoneoscopy revealed a normal-sized liver without obvious infiltration or other defects. Percutaneous liver biopsies revealed mild centrilobular cholestasis involving hepatocytes and, occasionally, bile canaliculi, with areas of slightly increased leukocytes, focal minimal hepatocellular degeneration, and mild hemosiderin and lipofuscin pigmentation (fig 1).

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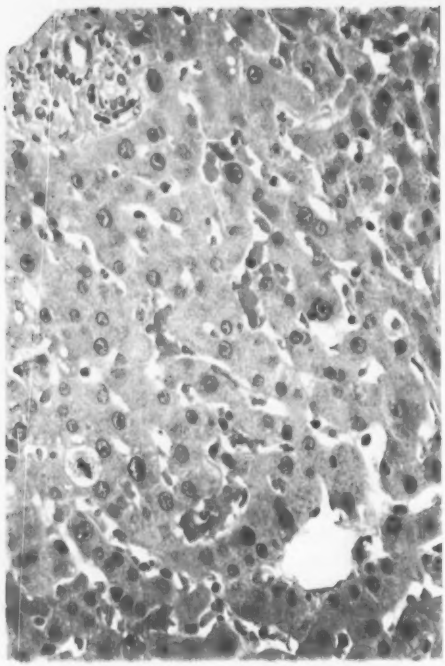

FIGURE 1.—Liver biopsy section from patient 1. Mild centrilobular cholestasis is present; other changes are minimal. Hematoxylin and eosin stain, × 250.

Although therapy was discontinued, the bilirubin (maximum, 7.9 mg/dl total; direct fraction, 5.6 mg/dl) and alkaline phosphatase (maximum, 1700 mU/ml) remained elevated for > 2 months while the SGOT decreased to near normal levels. The patient had disease progression and died; there was no autopsy.

Patient 2

Patient 2, a 58-year-old male, was treated with cobalt radiotherapy in 1977 for an adenocarcinoma of the lung. In January 1978, he presented with progressive disease in the chest and started to receive chlorozotocin, 225 mg/m² iv, as a single 360-mg total dose every 6 weeks. He tolerated chemotherapy well but required dose reductions at the sixth and seventh courses because of leukopenia. Following the sixth course of chlorozotocin in September 1978, he complained of anorexia associated with significant weight loss. During this time he had modest transient elevations of alkaline phosphatase to 316 mU/ml, of SGOT to 48 mU/ml, and of direct bilirubin to 0.6 mg/dl (total, 0.8 mg/dl). Following his seventh dose of chlorozotocin, he was re-admitted to the hospital because of continued anorexia and weight loss. He denied other complaints, and the initial physical examination was unremarkable with regard to the liver.

Admission laboratory studies included a stable hemoglobin concentration of 11.2 g/dl, wbc count of 10,500/μl with no eosinophils, and a platelet count of 60,000/μl. The alkaline phosphatase was elevated at 142 mU/ml, but the SGOT was normal at 25 mU/ml, and the total bilirubin was 0.6 mg/dl, with a direct fraction of 0.4 mg/dl. The other laboratory studies were within the normal range, including a negative HbSAg on two separate occasions. Within 2 days, liver function test values began to increase, with peak levels of alkaline phosphatase of 190 mU/ml and SGOT of 112 mU/ml occurring 4 days later. Bilirubin was also elevated at 2.4 mg/dl and 1.6 mg/dl, respectively, for the total and direct fractions. Medications during this time included multiple vitamins, aminophylline, isoetharine, acetaminophen, and hyperalimentation fluids (Intralipid and Freamine II). The patient subsequently developed a bronchopneumonia and progressive respiratory failure culminating in his death 10 days later. At death, his bilirubin increased to a maximum of 12.7 mg/dl, with a direct fraction of 9.3 mg/dl. The SGOT and alkaline phosphatase remained minimally elevated. During his acute illness, he received several medications including penicillin, gentamicin, amikacin, furosemide, diazepam, and cimetidine. However, the onset of the liver function abnormalities preceded the start of therapy with these agents.

At autopsy, the liver had prominent centrilobular cholestasis (fig 2) and was without evidence of tumor infiltration. Microscopic examination showed moderate centrilobular cholestasis, predominantly in bile canaliculi but also in hepatocytes and Kupffer's cells. There was focal mild hepatocellular degeneration, Kupffer's cell hyperplasia, and hemosiderin pigmentation but no evidence of hepatitis or neoplasm (fig 3).

Patient 3

Patient 3, a 48-year-old male, was diagnosed as having adenocarcinoma of the lung in August 1979. The initial evaluation revealed only regional node metastases. The liver was negative for metastatic disease by radionuclide scan and sonography. Serum alkaline phosphatase, SGOT, SGPT, and bilirubin were all within normal limits. The patient received a single 337-mg total dose of chlorozotocin (225 mg/m² iv) on September 7, 1979. At 4 weeks there were mild elevations in alkaline phosphatase and SGOT

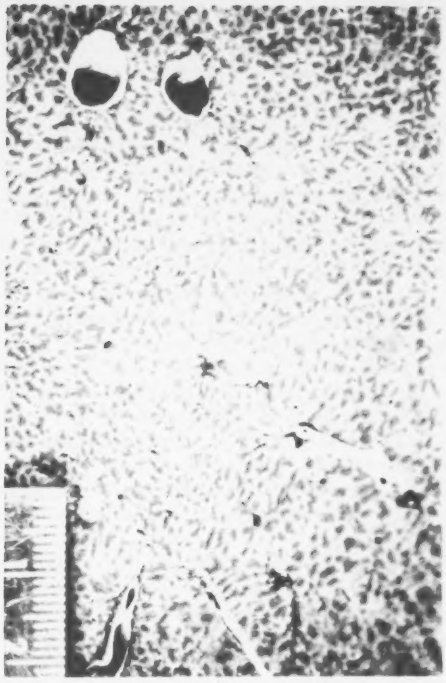

FIGURE 2.—Liver cut surface at autopsy from patient 2. Prominent centrilobular cholestasis is present.

but not in bilirubin. Over the next 6 weeks there were marked elevations in alkaline phosphatase (maximum value, 3157 IU/liter), SGPT (1395 IU/liter), and bilirubin (maximum, 3.5 mg/dl total; 3.0 mg/dl direct), with only a mild elevation in the SGOT (maximum, 137 IU/liter). Other studies were unremarkable. Specifically, the hemoglobin concentration was stable, and there was no eosinophilia. Neither fever nor dermatitis was noted, and physical examination showed that the liver was not enlarged. Abdominal sonogram revealed a slightly enlarged gallbladder, but there was no evidence of calculi, and the bile ducts were not dilated. There were no focal defects in the liver, either by sonography or radionuclide scan. Other medications taken during this period included multivitamins, acetaminophen, viokase for chronic ethanol-induced pancreatic insufficiency, occasional chloral hydrate, and a single 10-mg dose of prochlorperazine given orally just prior to administration of chlorozotocin. The patient denied recent intake of alcohol.

Percutaneous liver biopsy revealed moderate cholestasis involving bile ducts, canaliculi, and occasionally, hepatocytes and Kupffer's cells, without particular lobular localization. Other abnormalities were only minimal in degree and included fatty metamorphosis, focal portal fibrosis, and hemosiderosis.

Subsequently, the patient had progressive disease in the lungs, developed pneumonia, and died; there was no autopsy.

DISCUSSION

Drug-induced hepatotoxicity is commonly divided into two types, cholestatic and hepatic, although features of both may be seen in the same patient (9). Previous reports of hepatic lesions due to antineoplastic agents have emphasized a predominance of hepatocellular necrosis (9,10). Cholestasis, when present, was invariably associated with hepatic cell damage (6,8).

Hepatotoxicity has been reported following treatment with nearly all of the nitrosoureas used in clinical trials (1–8). Toxicity is manifested primarily by transient elevations of serum enzymes and jaundice and may be seen in up to 26% of patients treated. Pathologic examinations of autopsy specimens have demonstrated hepatocellular necrosis in some instances (4), while at other times no significant pathologic changes were detected (7).

Chlorozotocin is a glucopyranose-substituted nitrosourea structurally related to the antitumor agent streptozocin. Of the nitrosoureas, chlorozotocin possesses the most potent alkylating properties.[4] Preclinical studies in dogs showed reversible hepatic toxicity consisting of elevated serum enzymes and bilirubin, with pathologic changes of congestion and fatty deposition.[4] Phase I trials of chlorozotocin revealed transient elevations of serum SGOT and SGPT in one fourth to one third of the patients treated with single doses > 120 mg/m^2 (11,12). The hepatotoxicity described with single intermittent doses of chlorozotocin was not seen when the same total dose was administered over a 5-day period (13). The occurrence of cholestatic jaundice in three patients treated with chlorozotocin appears to be one of the few reported instances of drug-induced, predominantly cholestatic hepatitis due to an antineoplastic agent.

The etiology of chlorozotocin-induced hepatic toxicity was studied in mice by Woolley and Wang (14). They concluded that hepatic injury was related in part to drug metabolism by the mixed-function oxidase system and glutathione conjugation of chlorozotocin and/or its metabolites. The latter point

[4]Helman LJ, and Slavik M. Clinical brochure: chlorozotocin. Prepared by the National Cancer Institute, Bethesda, MD, Feb 1976.

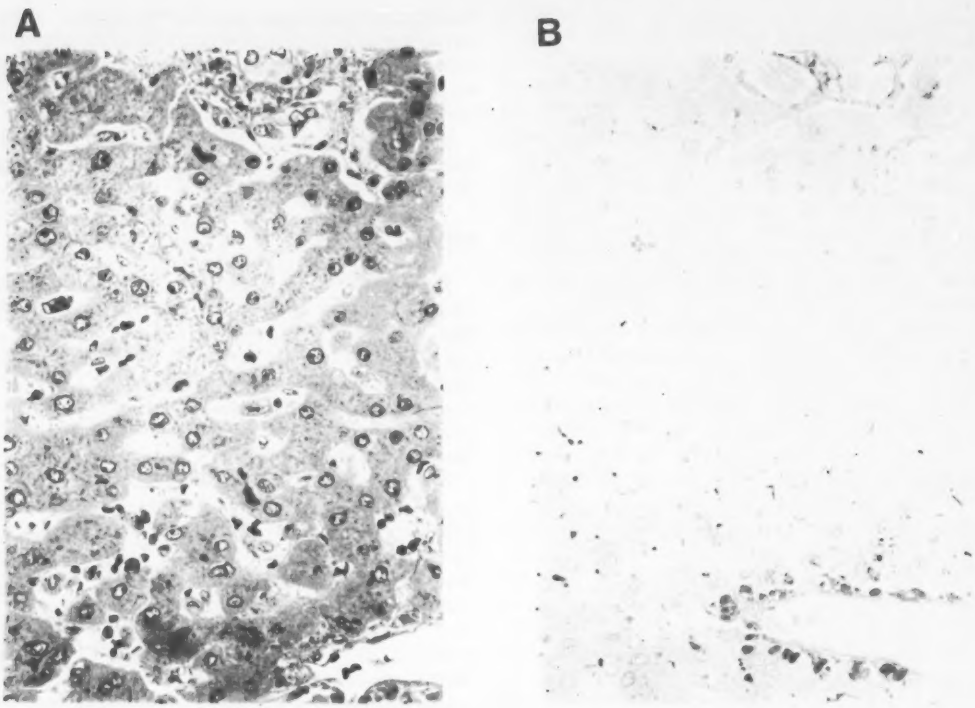

FIGURE 3.—Liver section from patient 2. Moderate centrilobular cholestasis is present and the centrilobular distribution of the dark-staining bile is accentuated in the section stained by Hall's method for bilirubin; mild nonspecific reactive and degenerative changes are also present. A. Hemotoxylin and eosin stain, × 250. B. Hall's stain, × 250.

may be especially important since the three patients in our study may well have had depleted glutathione stores due to debilitation and treatment with acetaminophen (15,16). It should be noted, however, that the pathologic changes seen in the livers of animals treated with chlorozotocin were predominantly those of hepatocellular injury (14).[4] Since the mechanism of drug-induced intrahepatic cholestasis is unknown, considerations regarding the etiology of chlorozotocin-induced cholestasis at present are entirely speculative.

The specific causes of pathologic changes seen in patients with cancer are often difficult to determine due to the complexity of the disease and the multiplicity of drugs administered. None of these patients had metastatic disease in the liver, nor was there any abnormality of liver function noted prior to treatment with chlorozotocin. Patient 1 was receiving only multiple vitamins and acetaminophen in addition to chlorozotocin. Although acetaminophen has been associated with hepatotoxicity, the pathologic picture is usually that of hepatocellular necrosis and is generally seen with repeated or high-dose administration (15). In the case of patient 2, several facts tend to support the contention that chlorozotocin caused the intrahepatic cholestasis. First, transient elevations in serum enzymes and direct bilirubin were seen several weeks prior to the terminal illness, at a time when chlorozotocin was the only medication being given. Additionally, initial increases in serum enzymes and bilirubin were documented prior to administration of the other drugs known to produce cholestatic jaundice. Finally, the 10-day interval between start of therapy with the other agents in question and the patient's death is unusually brief for these agents to produce the clinical and pathologic changes noted. However, the possibility of additive hepatotoxic effects from one or more of the drugs known to produce cholestatic jaundice cannot be excluded. In patient 3, cholestasis due to chlorozotocin was suspected because of knowledge of the two previous

cases. The patient had a history of ethanol abuse, but there was no evidence of alcohol intake for several weeks prior to receiving chlorozotocin. However, this patient had received prochlorperazine, an agent known to produce cholestatic jaundice. Phenothiazine-induced cholestasis, however, is usually more abrupt in onset and is often accompanied by evidence of a hypersensitivity reaction such as eosinophilia or dermatitis, neither of which was found in this patient (9). The etiology of hepatotoxicity in this patient cannot be determined with certainty and may have been caused by chlorozotocin, prochlorperazine, or both.

Several features of chlorozotocin-induced cholestasis should be emphasized. All three patients were treated with high, intermittent doses. Two of the patients had received four or more courses, suggesting that cholestasis may be a cumulative effect of the drug. However, peak drug levels of chlorozotocin may also be important in producing intrahepatic cholestasis. Support for the possibility that peak levels produce cholestasis includes the fact that all three patients received doses that were higher than the maximum doses administered in previous series and recommended by some investigators (12,13); the absence of hepatotoxicity with the daily × 5 schedule, compared with the single-dose schedule (13); and the occurrence of cholestatic jaundice in patient 3 following a single high dose of chlorozotocin. Conclusions regarding the association between cholestasis and peak levels of chlorozotocin in patient 3 must be tempered by the possible causative or additive effects of prochlorperazine.

Other features included the absence of fever, dermatitis, eosinophilia, or other evidence of a sensitization-type reaction; all three patients were essentially asymptomatic. While one patient had drug-induced transient liver function abnormalities prior to the development of intrahepatic cholestasis, cholestatic jaundice was the first clinical evidence of liver dysfunction in the other two patients. In the one evaluable patient, clinical manifestations of cholestasis persisted for the remaining 2 months of his life, even though therapy with chlorozotocin was discontinued.

The incidence of cholestatic hepatotoxicity from chlorozotocin or other nitrosoureas is unknown since the majority of patients with transient elevations in serum enzymes of bilirubin do not have pathologic confirmation by liver biopsy. The identification of chlorozotocin-induced cholestasis is important for at least two reasons. First, there are a multitude of possible causes for jaundice in patients with cancer. Second, there may be increased toxicity from combination chemotherapy with agents such as doxorubicin which are excreted in the biliary tract.

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Extensive Adenocarcinoma and Large Cell Undifferentiated Carcinoma of the Lung Treated With 5-FU, Vincristine, and Mitomycin C (FOMi)[1,2]

Thomas P. Miller,[3,*] Leo J. McMahon,[3,†] and Robert B. Livingston[4,5]

SUMMARY

Fifty-six patients with extensive (52 with TNM stage III M_1 disease and four with TNM stage III M_0 disease) adenocarcinoma (46 patients) and large cell undifferentiated carcinoma (ten patients) of the lung were treated with combination chemotherapy consisting of 5-FU, vincristine, and mitomycin C (FOMi). The patients had not received prior chemotherapy. The overall response rate was 41% (23 of 56 patients). Four patients achieved a complete response and 19 achieved a partial response. In 12 patients the disease was stable. Response did not vary by cell type: adenocarcinoma, 18 of 43 patients (42%); large cell carcinoma, four of ten (40%); and alveolar cell carcinoma, one of three (33%). The response varied by initial performance status. For a Karnofsky score of $\geq$ 70%, 20 of 41 patients (49%) responded, while for a Karnofsky score of $<$ 70%, three of 15 patients (20%) responded ($P = 0.22$). Survival was improved for responding patients regardless of initial performance status. The median survival duration was 24 weeks for the entire group of patients treated with FOMi. Survival of the responders (complete response plus partial response) was significantly improved over that of patients with progressive disease (28 weeks versus 13 weeks, respectively; $P = 0.002$). The FOMi combination was very well-tolerated. Nausea was common, but vomiting occurred in only four of 56 patients. Thrombocytopenia, requiring a reduction in the mitomycin C dose, developed after the third treatment course in 14 patients.

[Cancer Treat Rep 64:1241–1245, 1980]

Recently, several studies have reported gains in the treatment of non-small cell carcinoma of the lung as gauged by response rate. These recently reported drug regimens most frequently include doxorubicin (Adriamycin) and cyclophosphamide (1–5). We are reporting the results of the combination of 5-FU, vincristine, and mitomycin C (FOMi) in the treatment of metastatic adenocarcinoma and large cell undifferentiated carcinoma of the lung.

PATIENTS AND METHODS

Between December 1977 and April 1978, 58 patients with biopsy-proven, unresectable adenocarcinoma (46 patients) or large cell undifferentiated carcinoma (12 patients) of the lung were treated with FOMi at the Tucson Veterans Administration Medical Center, the San Antonio Veterans Administration Medical Center, and the University of

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Arizona Hospital. Of the 58 original patients, 56 were evaluable, one died at Day 13, and one was lost to followup. Histologic cell types were categorized according to the WHO classification scheme (6). There were 49 males and nine females, with a median age of 61 years (range, 47–78). Seven patients were > 69 years old.

All patients had measurable disease. Patients were staged using the TNM system (7). Fifty-four patients had stage III M_1 disease and four patients had stage III M_0 disease with documented progression of disease in a previously irradiated field. No patient had previously received chemotherapy and none had received concomitant radiotherapy. Seven patients had brain metastases and received irradiation to the brain immediately prior to the initiation of chemotherapy. Patients receiving prior irradiation to ports outside the brain were eligible for the study only if the disease was shown to progress following completion of radiation.

Forty-two patients received the FOMi regimen every 3 weeks for three treatment courses, and thereafter, every 6 weeks. For these 42 patients, 5-FU was administered at a dose of 300 mg/m²/day as a bolus on Days 1–4. Vincristine (2 mg) and mitomycin C (10 mg/m²) were both given on Day 1 of each treatment course. For patients who had difficulty returning to the hospital, 5-FU was given orally on Days 2–4. Otherwise, all drugs were given as an iv bolus. Sixteen patients received the FOMi regimen every 4 weeks with the 5-FU given as a continuous infusion (1 g/m²/day) on Days 1–4, vincristine given as an iv push (2 mg) on Day 1, and mitomycin C (10 mg/m²) given iv on Day 1. Appropriate reductions in dose were made if significant myelosuppression occurred. Patients were treated until disease progression.

Initially abnormal studies were repeated periodically to evaluate response. A complete response (CR) was defined as the disappearance of all measurable disease. A partial response (PR) was defined as a > 50% reduction in the product of two perpendicular tumor diameters of all evaluable lesions without evidence of progression in other sites. Stable disease was defined as a < 50% reduction and no increase in tumor dimensions lasting a minimum of 6 weeks. Progressive disease was defined as any increase in tumor dimensions.

The actuarial method of Kaplan and Meier (8) was used to calculate survival. Comparisons were made using Gehan's modification (9) of the Wilcoxon two-tailed test.

RESULTS

The objective response rate (CR plus PR) was 41% for the 56 evaluable patients. There were four CRs and 19 PRs. In addition, ten patients had stable disease. The response by cell type is summarized in table 1. It is of interest that three patients with the most poorly differentiated large cell carcinomas all responded to chemotherapy.

Survival for this group of patients is shown in figure 1. The median survival of the entire group was 24 weeks, with a median followup of 50 weeks. Eleven patients remain alive. Twenty-four patients with progressive disease had a median survival of 13 weeks. One patient with progressive disease is alive at 26+ weeks. Twenty-three patients showing objective responses (CR plus PR) to therapy have a median survival of 28 weeks. Eight patients with objective remissions are alive. The longest survivor is free of detectable disease at 108+ weeks. The survival advantage for patients who respond to chemotherapy compared with patients who have progressive disease is significant ($P = 0.002$). Ten patients were considered to have stable disease, with a group median survival of 25 weeks. Two patients with stable disease are alive. Importantly, patients with stable disease have significantly improved survival over patients with progressive disease ($P = 0.002$). At this time there is not a sig-

TABLE 1.—Response vs histologic subtype

	All patients	Adenocarcinoma	Large cell undifferentiated carcinoma	Alveolar cell carcinoma
No. of patients				
Total	56	43	10	3
Stable disease	12	10	1	1
CR	4	3	1	0
PR	19	15	3	1
Response rate (%)	41	42	40	33
Median survival (wks)				
All patients	24	24	17	—
Responders	28	28	—	—

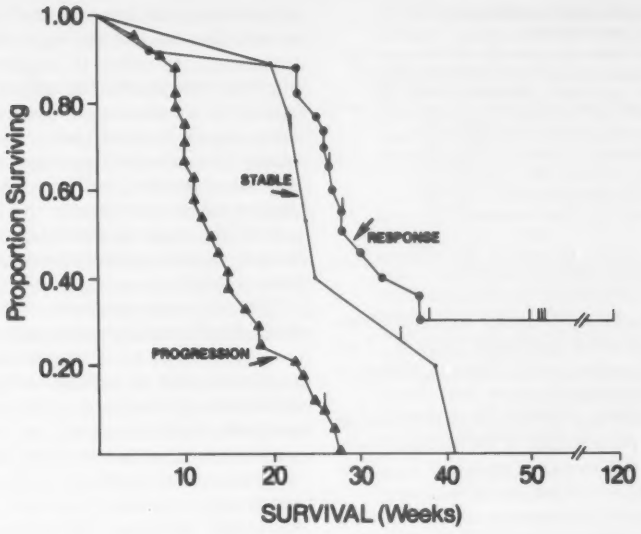

FIGURE 1.—Actuarial survival of 56 patients treated with FOMi.

nificant difference in survival between patients who respond and patients with stable disease ($P = 0.63$).

The response rate varied by initial Karnofsky performance status (table 2). In patients capable of self-care (Karnofsky score of ≥ 70%) the objective response rate was 48% (20 of 41 patients), while in patients with a Karnofsky score of < 70% the response rate was 20% (three of 15 patients). Survival duration was, however, improved at all levels of performance in responding patients (table 2).

The response rate did not vary with age. Three of seven patients > 69 years old responded to therapy. Weight change during the first 6 weeks of therapy with FOMi correlated with response. Only one of 24 patients with progressive disease gained weight during the initial 6 weeks of therapy. None of the four patients having CRs lost weight. Sixty percent of patients with PRs or stable disease gained or maintained their pretreatment weight. These changes in weight frequently preceded clinical response or disease progression.

The response rate was not significantly influenced by site of predominant disease involvement (table 3). Patients with bone disease responded to treatment infrequently. Only one of 11 patients with bone involvement responded to therapy in any site, while 22 of 45 patients without bone involvement responded objectively ($P = 0.085$). Seven patients had brain metastases. All of these patients had received whole-brain irradiation prior to initiating chemotherapy. All seven patients had measurable lesions outside the brain and responses were obtained in four of these. In spite of objective responses, the median survival of this group of patients was 12 weeks and is identical to the group of patients with progressive disease. Control of brain disease was achieved in five of seven patients; four of these patients responded and survived 8, 24, 26,

TABLE 2.—Response and survival by Karnofsky status

Karnofsky score (%)	No. of patients	No. of responders (rate)	Median survival in wks	
			All patients	Responders
90–100	14	9 (57%)	23	35
80–89	11	3 (27%)	23	27
70–79	16	8 (50%)	21	25
50–69	13	3 (23%)	10	28
0–49	2	0	—	—

TABLE 3.—Response by site of predominant disease involvement

Tissue involved	No. of responses/No. of patients treated (%)
Lung	13/31 (42)
Bone	1/11 (9)
Lymph nodes	6/8 (75)
Liver	2/5 (40)
Pleura	2/3 (67)
Soft tissue	1/2 (50)
Skin	1/1 (100)

and 28+ weeks. These responding patients eventually died of progressive systemic disease. Two patients with progressive brain disease failed to respond to FOMi systemically.

There was no significant difference in survival as a function of cell type (median of 24 weeks [adenocarcinoma] versus 17 weeks [large cell undifferentiated carcinoma], $P = 0.54$).

Although the drug combination was the same for all patients, 5-FU was administered by two different schedules. The response rate was 38% for 40 patients receiving bolus 5-FU whereas it was 50% for 16 patients receiving the drug as an infusion. This difference was not significant ($P = 0.5$).

The side effects of therapy were generally mild and well-tolerated. Nausea was common but vomiting was unusual (four of 56 patients). Myelosuppression was common, with prolonged thrombocytopenia (< 100,000 platelets/mm^3) developing after the third treatment course and requiring a reduction in the mitomycin C dose in 14 patients. Leukopenia (< 3000 cells/mm^3) developed in seven of 56 patients. There were two deaths due to septicemia in the presence of neutropenia. However, one of these patients was not treated for infection with antibiotics. These were the only episodes of fever associated with neutropenia. One patient developed a diffuse interstitial pattern on chest radiographs accompanied by progressive hypoxemia. This patient had received 85 mg of mitomycin C, but had not received previous radiotherapy. There were no significant differences in toxicity between routes of administration (infusion versus bolus) of 5-FU.

DISCUSSION

The initial treatment of non-small cell carcinoma of the lung depends on the extent of disease at diagnosis. In this respect, the TNM staging system as advocated by the American Joint Committee for Cancer Staging and End-Results Reporting has prognostic utility. Unfortunately, the vast majority of patients with carcinoma of the lung develop metastatic disease and die regardless of initial stage or treatment. In order to improve the overall 5-year survival rate of 8% (10), effective systemic therapy needs to be developed. Recently, several reports have shown modest gains, as gauged by objective tumor response, for patients receiving chemotherapy for non-small cell carcinoma of the lung. Response rates have been in the range of 35%–65% for adenocarcinoma and 35% for large cell carcinoma. These regimens most frequently use doxorubicin and cyclophosphamide (1–5).

The response rate in the current study compares favorably to results previously reported for patients receiving other drug regimens. Bitran et al (1) recently treated 22 patients with stage III M_1 adenocarcinoma of the lung with cyclophosphamide, doxorubicin, methotrexate, and procarbazine. There was a 36% response rate and improved survival for the responding patients compared to patients with progressive disease. Survival was not reported by histologic subtypes, but within the group of non-small cell carcinoma, responders lived a median of 12.6 months and patients with progressive disease lived a median of 2.4 months. Sixteen of 54 patients received radiation therapy to the lungs immediately prior to or concomitant with chemotherapy. Butler et al (2) report a 36% response rate in 25 patients with unresectable adenocarcinoma of the lung (TNM staging results were not recorded), with improved survival of responders (8.5+ months) over nonresponders (2.5 months); they used a three-drug combination of 5-FU, doxorubicin, and mitomycin C. Again, nine patients received prior or concomitant irradiation to the lungs. Because of the similar results obtained with 5-FU, doxorubicin, and mitomycin C and FOMi, any additional benefit ascribed to doxorubicin used with 5-FU and mitomycin C in this disease will need to be tested. Chahinian et al (3) have reported an impressive response rate of 58% in 26 patients with unresectable adenocarcinoma of the lung treated with methotrexate, doxorubicin, cyclophosphamide, and CCNU. In that same study, 20 patients with large cell undifferentiated carcinoma had a 35% response rate. In a subsequent cooperative group study, a much lower response rate (6%) was observed for methotrexate, doxorubicin, cyclophosphamide, and CCNU in 16 patients with adenocarcinoma of the lung (4). Combining the results gives a 38% response rate in 42 patients with adenocarcinoma. In both of these studies, approximately 20% of the patients had limited disease (TNM stage not available). Finally, Britell and co-workers (5) report that five of 19 patients with metastatic adenocarcinoma of the lung responded (PR plus CR) to cyclophosphamide, dox-

orubicin, and cisplatin; there was no apparent survival difference between responders and nonresponders.

It is difficult to compare drug regimens from previous studies of non-small cell carcinoma of the lung because of differences in the extent of disease, prior and concomitant therapy, and lack of result reporting according to histology. Comparisons could best be made in phase III group trials. However, the available information suggests that the FOMi regimen is comparable to previously reported doxorubicin- and cyclophosphamide-containing combinations with respect to response rate and survival duration. Furthermore, survival duration appears to be related to response with FOMi regardless of initial performance status. The combination is extremely well-tolerated, and hopefully, the FOMi regimen will prove to be non-cross-resistant to other combinations using doxorubicin and cyclophosphamide.

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Phase I Study of 6-Diazo-5-oxo-L-norleucine (DON)[1,2,3]

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SUMMARY

We conducted a phase I study of 6-diazo-5-oxo-L-norleucine given iv on a twice weekly schedule. Twenty-six evaluable patients received 31 courses of the drug. Doses ranged from 100 to 500 mg/m². Nausea with vomiting was the dose-limiting toxic effect, transient thrombocytopenia was seen frequently, and mucositis occurred in 39% of the patients. No definite therapeutic responses were observed in 18 patients with measurable lesions. The recommended dose for phase II studies is 200–300 mg/m² iv twice weekly.

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6-Diazo-5-oxo-L-norleucine (DON) is an antitumor antibiotic isolated from cultures of *Streptomyces* (fig 1). As a structural analog of L-glutamine, DON functions as a glutamine antagonist, interfering with the utilization of that amino acid in several key biochemical reactions (1).

The pharmacology and history of the use of the glutamine antagonists in the chemotherapy for malignant disease was extensively reviewed by Livingston et al in 1970 (2). Glutamine provides not only glutaminyl residues for the biosynthesis of peptides, but also donates an amido group for the synthesis of asparagine, nucleotides, and D-glucosamine. In at least several experimental systems, neoplastic tissues are reported to be deficient respectively in L-glutamine (3) and in glutamine synthetase (4), and to concentrate nitrogen-13 radiolabeled glutamine (5). Alterations in the availability of glutamine might selectively injure neoplastic cells while resulting in minimal toxicity to normal cells.

DON inhibits the pathways of purine (6), pyrimidine (7), phosphopyridine nucleotide (8), and D-glucosamine (9) biosynthesis. Furthermore, the drug inactivates glutamine synthetase in vitro (10). DON is an active cytotoxic agent with a broad spectrum of antitumor activity against rodent neoplasms (1).

Early clinical trials of DON were initiated in 1955. DON is rapidly cleared from plasma following iv injection; active drug, however, is not excreted in the urine. When given on a schedule of 0.1–0.3 mg/kg daily (parenterally or orally), considerable toxic effects, consisting primarily of stomatitis, diarrhea, nausea, and vomiting, were seen (11). Minor therapeutic activity was observed in patients with carcinoma of the breast (11), lung (11,12), uterus (11), and gastrointestinal tract (11); in patients with choriocarcinoma (13); and in patients with lymphosarcoma (11,12) and Hodgkin's disease (12). Extended disease-oriented phase II trials of DON as a single agent, however, were never conducted.

Azotomycin, another antagonist of glutamine, is a peptide consisting of two molecules of DON and one molecule of glutamic acid (fig 1); it is probably activated in vivo by conversion to DON (14). DON and azotomycin have nearly identical antitumor activity in murine tumor systems, with efficacy against L1210 and P388 leukemias, C26 and C38 colon tumors, and $CD8F_1$ mammary carcinoma, but not against Lewis lung carcinoma, intracranially im-

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[3]DON was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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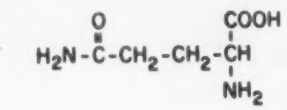
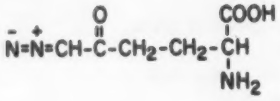
L-Glutamine



DON



Azotomycin

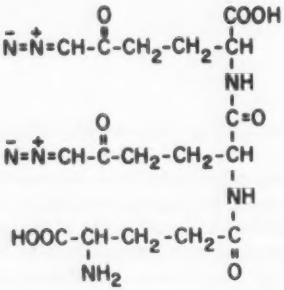


FIGURE 1.—Structure of L-glutamine, DON, and azotomycin.

planted ependymoblastoma, or B16 melanoma (15). Optimal antitumor effects of DON in murine tumor systems are schedule-dependent, with the best therapeutic index being achieved on a schedule of every 4 days (16). On that schedule, DON and azotomycin possess similar antitumor activity in human tumor xenografts transplanted to athymic mice (15). In human studies, azotomycin was found to have antitumor activity in colorectal carcinoma, soft tissue sarcoma, and mesothelioma (17). Although the results of the sarcoma trial could not be confirmed (18), the combination of azotomycin and 5-FU was shown to produce remissions in patients with colon cancer (19).

Because of the activity of DON in experimental tumor systems and the occasional responses to DON seen in human malignancies, disease-oriented phase II studies of DON are warranted. Further support for this approach is based on the reported activity of azotomycin in human colon cancer, the finding of the nearly identical antitumor activities of DON and azotomycin in murine tumors and human tumor xenografts, the putative activation of azotomycin by conversion to DON, and the difficulty with supplies of azotomycin (20).

In previous clinical trials, a daily dose schedule was used (11–13), although one patient in the study of Magill et al (11) was treated with 1.6 mg/kg of DON every 4 days. That patient experienced no stomatitis or myelosuppression, but did have nausea and vomiting. Since preclinical data show that such a schedule is optimal, further clinical trials should be done using an intermittent, rather than a daily, dose schedule. Prior to embarking on phase II studies, the present phase I study was conducted to identify an acceptable dose of DON and to define the toxic effects of the drug when it is administered twice weekly.

PATIENTS AND METHODS

Each patient treated had a histologically confirmed diagnosis of malignancy refractory to conventional modalities of treatment or for which no effective therapy is known. No patient had received radiotherapy or chemotherapy during the 4 weeks prior to treatment with DON. A performance status of at least 30% (Karnofsky scale), a wbc count $\geq 4000/mm^3$, a platelet count $\geq 100,000/mm^3$, stable cardiopulmonary status, normal serum bilirubin (< 1.5 mg/dl), and normal serum creatinine (< 1.5 mg/dl) were required for entry in this study. Informed consent was obtained from each patient prior to the initiation of treatment.

Each patient had an initial complete history and physical examination, chest roentgenogram, ECG, and urinalysis. The following baseline laboratory tests were performed for all patients: cbc, platelet and reticulocyte counts, electrolytes, creatinine, 5′-nucleotidase, prothrombin time, and a biochemical profile which included serum glucose, BUN, calcium, phosphorus, total protein, albumin, total bilirubin, alkaline phosphatase, LDH, and SGOT. Radionuclide scans were performed only when clinically indicated. An attempt was made to identify evaluable parameters of disease in all patients, but the absense of measurable lesions did not preclude entry in this study.

Initially, only hospitalized patients were treated; a cbc, platelet count, and biochemical profile were obtained at least three times weekly. As the study progressed and as experience was acquired, outpatient treatment was begun. A cbc and a platelet count were obtained prior to the administration of each dose of DON, and a biochemical profile was obtained weekly. Other diagnostic tests were ordered as clinically indicated.

DON was supplied in vials containing DON (100 mg), mannitol (100 mg), KH_2PO_4 (68 mg), and

NaOH to adjust the pH to 5.6. The drug was reconstituted with 5 ml of sodium chloride injection (USP), providing a solution with a DON concentration of 20 mg/ml. The drug was administered by rapid iv injection through a running iv line.

The planned course of treatment consisted of six doses of DON administered twice weekly over 3 weeks. Thereafter, patients were closely followed for at least 3 additional weeks before receiving further treatment with DON or other chemotherapy. The initial dose of DON, 100 mg/m² iv twice weekly, was chosen so that over the period of treatment the total dose administered would be slightly less than that tolerated in previous studies (11,12). The schedule of dose escalation was from 100 mg/m² to 150, 200, 300, 400, and 500 mg/m². The dose was not escalated within any course of treatment and at least three patients were treated at each level before other patients started treatment at the next dose level. However, several patients who had tolerated low doses of DON were re-treated at a higher dose level after an appropriate time interval.

A course of therapy was stopped if the patient developed myelosuppression, initially defined as a wbc count < 3000/mm³ or a platelet count < 100,000/mm³. After it became clear that recovery from thrombocytopenia was prompt, treatment was continued until the platelet count decreased to < 75,000/mm³. A course of therapy was considered evaluable if six doses of DON were given or if therapy was interrupted by hematologic toxicity or intolerable nausea or vomiting.

TABLE 1.—Characteristics of 26 evaluable patients

Characteristic	No. of patients
Sex	
Male	15
Female	11
Age in yrs	
Range: 36–73	
Median: 56	
Karnofsky performance status (%)	
30–50	13
60–70	11
80–100	2
Diagnosis	
Adenocarcinoma	
Breast	1
Colon	6
Gastric	1
Lung	4
Ovary	1
Unknown primary	2
Epidermoid carcinoma	
Bladder	1
Esophageal	1
Larynx	1
Uterine cervix	1
Hypernephroma	1
Hodgkin's disease	1
Non-Hodgkin's lymphoma	3
Sarcoma (soft tissue)	2
Prior therapy	
Radiotherapy only	2
Chemotherapy only	7
Chemotherapy + radiotherapy	17

RESULTS

Twenty-eight patients were entered in this study over 10 months. Two patients were not considered evaluable for this phase I study. One patient developed acute bacterial pneumonia not related to drug-induced myelosuppression; therapy with DON was stopped and never resumed because of clinical deterioration. Another patient was lost to followup after one dose of DON. Twenty-six patients received a total of 31 evaluable courses of DON. The characteristics of these patients are presented in table 1.

Toxicity

Nausea, vomiting, and myelosuppression were the dose-limiting toxic effects. The dose escalation schedule and the hematologic sequelae of treatment are shown in table 2. Myelosuppression was manifested primarily as thrombocytopenia, which generally occurred only after the third dose of treatment, although in one heavily pretreated patient with a lymphocytic lymphoma without demonstrable bone marrow involvement, the platelet count decreased from 195,000 to 63,000/mm³ after a single dose of DON (300 mg/m²). Recovery from thrombocytopenia was prompt, with return of the platelet count to within normal limits in 3–7 days from the day of the nadir. Occasional leukopenia was noted in heavily pretreated patients with poor performance status, but the degree of leukopenia was not clearly related to the dose of DON administered. There were no incidents of hemorrhage related to thrombocytopenia, and platelet transfusions were not required. No consistent pattern of decrease in hemoglobin concentration was observed.

With escalation of the dose, nausea and vomiting became more frequent and severe. Nausea generally began approximately 1 hour after drug administration, with vomiting occurring intermittently over the next 6–10 hours. However, when higher doses were used, patients complained of nausea persisting for 2–3 days. Because the drug was given twice weekly, patients at the highest dose level had

TABLE 2.—Hematologic toxicity with 31 courses of DON

Dose level (mg/m²)	No. of evaluable patients	No. of evaluable courses	Median No. of doses/ course	Wbc count nadir			Platelet count nadir		
				Range (× 10^3 cells/ mm³)	Median (× 10^3 cells/ mm³)	Day of median	Range (× 10^3 cells/ mm³)	Median (× 10^3 cells/ mm³)	Day of median
100	3	3	6	4.5–11.2	6.9	7	140–316	296	22
150	3	3	5	1.3–11.2	7.4	9	154–220	182	8
200	4	4	5	3.9–7.4	6.1	13	82–270	121	15
300	10	11	3	2.8–11.2	5.7	13	23–460	107	14
400	8	8	3	3.3–13.6	6.0	9	66–229	116	15
500	2	2	3	7.0–9.5	8.2	10	100–232	166	13

nearly constant nausea during the period of treatment. Patients at the higher dose levels tended to refuse treatment after fewer doses of DON than patients treated at the lower dose levels, because of severe nausea. The degree of thrombocytopenia observed was less profound as the dose was escalated above 300 mg/m², reflecting this tendency (table 2). Thus, at lower doses, the predominant dose-limiting toxicity was hematologic, whereas at higher doses, nausea and vomiting were the dose-limiting side effects. Since recovery from the thrombocytopenia was rapid, it appears that acute gastrointestinal toxicity was the true dose-limiting factor. The use of phenothiazines or hydroxyzine did not appear to influence the severity or duration of DON-induced nausea and vomiting.

Mucositis, primarily stomatitis, was seen in ten patients (39%). It was neither clearly dose-related nor severe; glossitis was noted in two patients who had received prior radiotherapy to the head and neck region. There were no other episodes which suggested radiation-recall phenomena. Occasional diarrhea, not clearly related to DON, did occur but there was no melena or hematochezia. No cutaneous toxicity or hepatic, renal, or cardiopulmonary complications of therapy were observed.

Therapeutic Responses

Eighteen of 26 evaluable patients had measurable parameters of disease. Definite antitumor activity was not noted in any of the patients in this study.

DISCUSSION

For a quarter of a century there has been interest in the use of antagonists of glutamine for the treatment of cancer. Although three such compounds (azaserine, DON, and azotomycin) have demonstrated antitumor activity in patients (21), extensive disease-oriented phase II trials of these drugs have not been conducted. The activity of DON and azotomycin in human tumor xenografts in nude mice and renewed interest in other drugs which interfere with amino acid metabolism (22) provided impetus for the clinical re-evaluation of DON. With evidence suggesting that the therapeutic index of DON would be improved by using an intermittent schedule of drug administration, this phase I study was initiated in anticipation of disease-oriented phase II trials.

The present study demonstrated that when given twice weekly, the dose-limiting toxic effects of DON are nausea, vomiting, and thrombocytopenia. Mild to moderate mucositis was seen frequently, but was not dose-limiting. Based on our findings, we recommend a 300-mg/m² dose of DON iv twice weekly for phase II studies in patients with good performance status. The dose should be attenuated to 200 mg/m² if nausea is intolerable at the starting dose. Therapy should be withheld if the platelet count is $< 75{,}000/mm^3$ or if the wbc count is $< 3500/mm^3$ and resumed when the blood cell counts recover. On this schedule, we would anticipate being able to administer three or four doses before interrupting therapy because of platelet count suppression. At least six to eight doses of the drug should be administered before a phase II trial of the drug is considered adequate.

Because of the reported activity of azotomycin in colon cancer, and because of the activity of azotomycin and DON in a human colon tumor xenograft, a phase II study of DON in this disease would be of particular interest. Regardless of the activity of DON as a single agent, the use of DON in combination with other antimetabolites or with glutaminase (23) holds promise for the control of tumor growth through the biochemical manipulation of neoplastic cell proliferation. By taking advantage of the differences in glutamine metabolism between neoplastic and normal tissues, an active combination with a high therapeutic index may be identified. Phase I studies of such combinations are currently under active consideration.

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Clinical Pharmacology of IMPY by Radioimmunoassay[1,2]

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SUMMARY

A radioimmunoassay for IMPY has been developed using [^{3}H]acetyl-IMPY and antibody induced by immunizing rabbits with succinyl-IMPY bovine serum albumin conjugates. The method was reproducible and sensitive at a dose of 0.2 μg/ml. The cross-reactivities of IMPY and IMPY derivatives with the antibody were compared. Using this radioimmunoassay, clinical pharmacology was performed in cancer patients during phase I trials. Following 30–60-minute iv infusions of IMPY (450–1500 mg/m^2/day for 5 days), the drug disappeared rapidly from plasma with an initial half-life of < 10 minutes and a second half-life of 4 hours. The half-lives are not affected by minimally abnormal hepatic function and are not dose-related. The apparent volume of distribution was approximately 125 ml/kg. The results obtained on Day 5 were essentially the same as those obtained on Day 1. IMPY was also detected in glioblastoma, brain, and temporalis muscle when the drug (500 mg/m^2) was infused iv 4–5 hours before surgery. The tissue drug levels were comparable to or higher than those of plasma. The drug was also measurable in kidney and liver tissues obtained 4 hours after death in a patient who died 21 hours after iv infusion of IMPY at a dose of 1500 mg/m^2/day for 3 days.

[Cancer Treat Rep 64:1253–1260, 1980]

IMPY rapidly and selectively inhibits DNA synthesis, but does not inhibit RNA or protein synthesis in cultured mammalian and mouse leukemic cells (1,2).[5,6] Cells treated with IMPY can also be synchronized at the point of entry into the S phase.[6] The DNA synthesis and replication cycle were rapidly resumed when the IMPY-treated cultures were restored to drug-free medium.[6] Brockman et al (3) showed that IMPY, similar to hydroxyurea and guanazole (Gz), inhibits ribonucleotide reductase in vitro. However, the duration of DNA synthesis inhibition by IMPY in L1210 cells in vivo is much longer than that by hydroxyurea or Gz (3). IMPY is active against ip implanted mouse L1210 leukemia when the drug is administered ip, sc, or orally. It is also active against mouse L1210 leukemia variants that have been developed for resistance to BIC, DTIC, cytosine arabinoside, thiosemicarba-

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zone, and Gz.[5,6] For these reasons, IMPY has been under clinical study at the Ohio State University Cancer Center, Columbus (4) and The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston (5). Using 7-bromo-IMPY as an internal standard, a sensitive electronic-capture gas chromatographic assay for IMPY was employed for pharmacokinetic study (4,6), and the metabolic products in dogs have been reported (7). The drug disposition has also been studied in mice using ^{14}C-labeled IMPY (8). We have developed a radioimmunoassay for pharmacologic studies while IMPY is in phase I trials; a preliminary report has appeared elsewhere (9).

MATERIALS AND METHODS

Materials

IMPY was supplied by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Tritiated acetic anhydride (specific activity, 3.1 curies/mmol) was purchased from Amersham/Searle Corp, Arlington Heights, IL. *N*-β-Ribosyl-IMPY was kindly provided by Dr. L. Malspeis, Ohio State University Cancer Center. Dextran T70 was purchased from Pharmacia, Uppsala, Sweden. Norit A charcoal was obtained from Fischer Scientific, Fair Lawn, NJ. Silica gel plates (silica gel F254) were purchased from E. Merck, Darmstadt, W Germany. Succinic anhydride, bovine serum albumin (BSA), Freund's complete adjuvant, *N*-hydroxysuccinimide, and other chemicals were commercially available. Female New Zealand rabbits, approximately 2.5 kg and 12–15 weeks of age, were purchased from Rich-Glo Co, Houston, TX.

Elemental analyses were performed by Atlantic Microlab, Inc, Atlanta, GA. Melting points were determined on a Uni-Melt (Thomas Co, Philadelphia, PA) and were corrected. Infrared was obtained with a Perkin-Elmer 727B spectrophotometer.

Preparation of Antigen (fig 1)

Synthesis of succinyl-IMPY.—IMPY (300 mg, 2.7 mmols) was added to a solution of succinic anhydride (324.2 mg, 3.24 mmols) in 8 ml of glacial acetic acid. The reaction mixture was stirred at ambient temperature under anhydrous conditions for 18 hours. A white solid precipitated out of the reaction mixture and was collected by filtration (286.8 mg). The filtrate was evaporated to a white powder which was stirred for 15 minutes in 60 ml of hot ethyl acetate. The insoluble material was filtered to obtain another crop of white powder (219 mg). The two crops had identical melting points (215°C–216°C) and infrared (KBr), 3500 (OH), 1740 (carboxylic acid), and 1675 cm^{-1}(amide). The total yield was 505.8 mg, 89.5%. Analysis calculated for

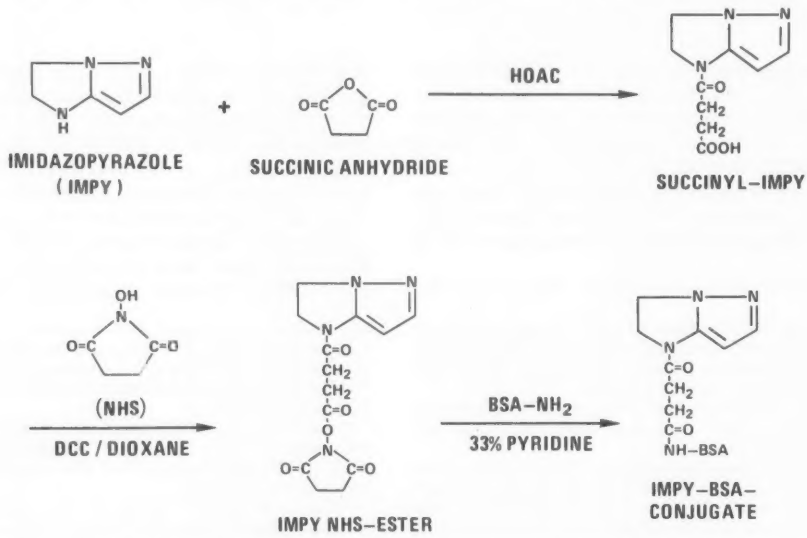

FIGURE 1.—Preparation of antigen. NHS = *N*-hydroxysuccinimide; DCC = dicyclohexylcarbodiimide; HOAC = acetyl acid.

$C_9H_{11}N_3O_3$ was: C, 51.67; H, 5.35; N, 20.08; analysis found: C, 51.74; H, 5.35; N, 20.04.

Conjugation of succinyl-IMPY and BSA.—Succinyl-IMPY (31.3 mg, 0.15 mmol), *N*-hydroxysuccinimide (17.8 mg, 0.15 mmol), and dicyclohexylcarbodiimide (30.6 mg, 0.15 mmol) were dissolved in 4 ml of dried dioxane. The resulting solution was stirred at ambient temperature for 3 hours under anhydrous conditions and then kept at 4°C overnight. The precipitated side product, dicyclohexylurea, was removed by filtration and washed with dioxane. The combined filtrate was then evaporated to dryness, dissolved in 3 ml of pyridine, and cooled in an ice bath. BSA (8.8 mg in 1.5 ml H_2O) was slowly added to this cooled solution, and the resulting solution was stirred for 3 hours at ambient temperature. The reaction mixture was then dialyzed against 1 liter of 50% pyridine-H_2O and 30% pyridine-H_2O for 4 hours each, and followed by H_2O for 2 days with three changes per day. The dialyzed mixture was then lyophilized to yield 8.9 mg of the succinyl-IMPY-BSA conjugate as a white powder. The thin-layer chromatography (TLC) (silica gel) of the product showed one single spot at the origin as detected by ultraviolet light and iodine. Using the trinitrobenzenesulfonic acid method for determination of primary amine, the conjugate was estimated to contain 20 succinyl-IMPY residues per BSA molecule (10).

Immunization

Succinyl-IMPY-BSA conjugates in 0.9% NaCl (2 mg/ml) were emulsified with an equal volume of Freund's complete adjuvant. Three rabbits were immunized with 1 ml each of the emulsion injected at multiple intradermal and subcutaneous sites. Booster injections (0.5 ml) were given at biweekly intervals. After 3 months of immunization, each rabbit was bled from an ear vein 1 week after the booster injections. The plasma was separated by centrifugation and stored at –80°C, and IMPY antibody was demonstrated in the rabbit's plasma.

Preparation of *N*-Acetyl-IMPY

IMPY (30 mg, 0.28 mmol) was dissolved in a mixture of acetic anhydride (30.6 mg, 0.30 mmol) and 2 ml of glacial acetic acid. The resulting solution was incubated at ambient temperature for 20 hours with protection from moisture and light. After lyophilization, a white powder (42.2 mg, 100%) was obtained and shown to be homogeneous by TLC; melting point, 108°C–109°C [reported (11) melting point, 111°C–112°C], infrared (KBr), 1680 cm^{-1} (amide).

Preparation of *N*-Formyl-IMPY

Acetic anhydride (0.85 ml) was slowly added to a solution of IMPY (200 mg, 1.83 mmols) in 88% formic acid (2.5 ml). The solution was warmed at 50°C–60°C for 15 minutes and then stirred at ambient temperature for 2 hours. The excess formic acid and acetic anhydride were evaporated and gave the crude product as a yellow crystal solid (240 mg, 95.6%), infrared (KBr), 1660 cm^{-1}(amide). Analytic sample was prepared by recrystallization from ethyl acetate, melting point, 78°C–79°C. Analysis calculated for $C_6H_7N_3O$: C, 52.55; H, 5.18; N, 30.64; analysis found: C, 52.53; H, 5.18; N, 30.60.

Radioimmunoassay

The assay was performed in 3-ml glass tubes containing 100 µl each of [^{3}H]acetyl-IMPY with approximately 7000 cpm, 0.01 M phosphate-buffered saline (PBS) (pH 7.5), antiserum (diluted 1:20 with 0.1% gelatin in 0.01 M PBS), and an appropriate amount of IMPY standard or unknown samples in a total volume of 0.5 ml. The binding reaction was initiated by the addition of antiserum and incubated at room temperature for 1 hour. The tubes were then placed in an ice bath and a 0.5-ml solution of 0.5% dextran and 1% Norit A charcoal in 0.01 M PBS was added. After the tubes were allowed to stand in the bath for 10 minutes, the mixture was centrifuged in an International PR2 centrifuge at 900 × *g* for 15 minutes at 5°C. A 0.5-ml aliquot was added to 10 ml of 66% Phase Combining System counting solution (Amersham/Searle Corp) in xylene and measured for radioactivity in a Packard scintillation spectrometer, Model 3375. The relative counting efficiency for the ^{3}H was 35%, and any quenching was corrected by the channel ratios with an automatic external standard. Data points for the standard curve and unknown samples were obtained in duplicate.

Pharmacology

One female and nine male patients with metastatic cancer were included in the study. IMPY (450–1500 mg/m²/day for 5 days) was infused iv over 30 or 60 minutes. Blood samples were drawn and heparinized before and after infusion at designated time intervals. Plasma was separated by centrifugation and immediately frozen until assayed. An attempt was made to study the IMPY metabolites and test for their cross-reactivities against the anti-IMPY serum. Plasma samples were extracted with 3 volumes of methylene chloride. The organic layer was evaporated to dryness under nitrogen and re-

constituted with chloroform; 10 μl of the chloroform extract was analyzed for IMPY and its metabolites by gas chromatography with an OV17 column. Injector and flame ionization detector settings were 280°C, with a nitrogen flow of 40 ml/minute and attenuation of 4×10^{-9} A. The column was first run isothermally for 6 minutes at 110°C followed by a programmed temperature increase of 5°C/minute to 150°C, and then was held at 150°C an additional 6 minutes. Tissue samples were obtained during autopsy and surgery. Liver and kidney tissues were obtained from one patient who died 21 hours after IMPY infusion (1500 mg/m²/day × 3) and was autopsied 4 hours after death. Plasma was obtained 18 hours after the iv infusion, or 3 hours before death. Another patient received a 60-minute iv infusion of IMPY at 500 mg/m², 4 hours prior to surgical resection of a glioblastoma multiforme. Samples of tumor, brain, plasma, and temporalis muscle were also obtained 5 hours after drug infusion. The tumor was divided into central and peripheral portions which were analyzed separately. Tissues were homogenized in 2 volumes of ice-cold distilled H_2O, and perchloric acid was added to a final concentration of 4%. After standing in ice for 15 minutes, the denatured protein was removed by centrifugation, and the supernatant was neutralized to pH 7.0 with KOH. The precipitated potassium perchlorate was removed by a brief centrifugation and the supernatant was assayed for the drug.

RESULTS

Standard Curve

The ability of unlabeled IMPY, ranging from 0.1 to 2 μg, to compete for the binding of [³H]acetyl-IMPY to the antisera is shown in figure 2. A linear curve is obtained by plotting the percent of binding inhibition versus IMPY concentration on logit-log paper.

The specificity of the IMPY antisera was tested with several IMPY derivatives. It was found that the antibody cross-reacts with *N*-succinyl-, *N*-acetyl-, *N*-formyl-, and *N*-β-ribosyl-IMPY with 50% binding inhibitory concentrations of 0.0029, 0.0035, 0.28, and 19.2 μg/0.5 ml assay volume, respectively (fig 3).

Pharmacology

Patients received IMPY doses ranging from 450 to 1500 mg/m²/day for 5 days. Blood was sampled at designated time intervals on Day 1 and if possible, also on Day 5. A typical example of the plasma

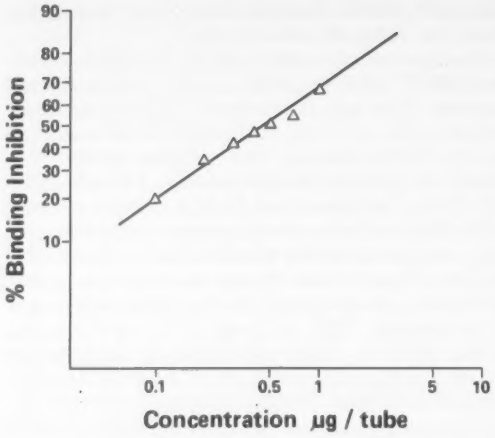

FIGURE 2.—IMPY standard curve by radioimmunoassay.

disappearance curve is shown in figure 4. The initial plasma half-life was short: < 10 minutes. The terminal half-life was approximately 4 hours. The data obtained on Day 5 were essentially the same as on Day 1 (fig 4 and table 1). It is also of interest to note that the samples assayed on different days showed similar results (fig 4). The half-life was not affected by minimally abnormal hepatic function and was not dose-related (table 1). The apparent volume of distribution was 125 ± 46 (ml/kg ± SD). The initial plasma level is well-correlated with the dose administered (fig 5); the higher the dose, the higher the initial plasma concentration.

The drug was also detectable in brain, glioblastoma, and temporalis muscle 4–5 hours after IMPY was given to patients (fig 6). The drug was evenly distributed in the center and periphery of the tumor and its level in tissues was comparable to that in plasma (fig 6). As shown in figure 7, the drug was also measurable 4 hours after death in kidney and liver samples of a patient who died 21 hours after the third daily dose of IMPY. Three compounds in several initial plasma samples were found by the gas chromatographic separation, and they had the same retention times as authentic IMPY, *N*-acetyl-IMPY, and *N*-formyl-IMPY. Samples at later hours, however, contained more than seven peaks.

DISCUSSION

We have developed the radioimmunoassay for IMPY to perform the clinical pharmacology. Using this assay, the pharmacokinetics of IMPY were studied in seven cancer patients during phase I

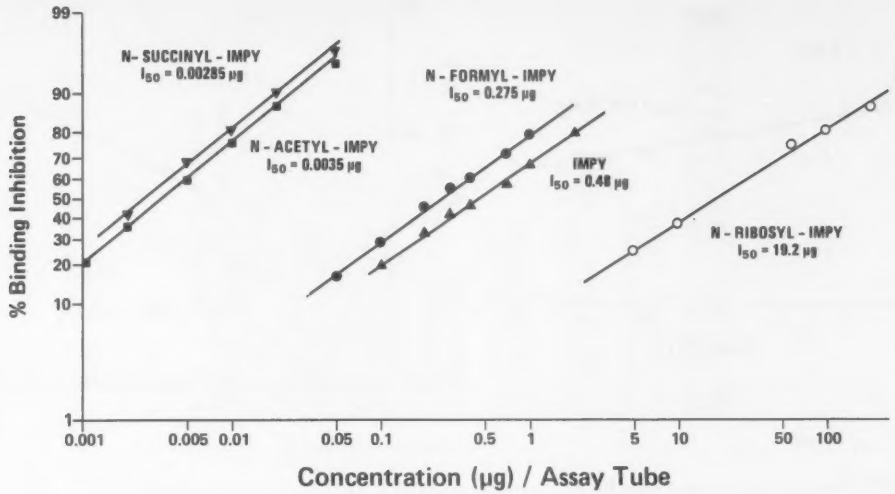

FIGURE 3.—Comparison of cross-reactivities of IMPY and IMPY derivatives with antibody.

trials. The method is simple and, more important, is reproducible, as shown by the similar results for the samples assayed on different days (fig 4).

IMPY coupled to BSA through a conjunction with succinic acid was used to induce antibody in rabbits. [^{3}H]acetyl-IMPY was used as the labeled ligand in the assay. The antibody specificity was determined by measuring the displacement of bound [^{3}H]acetyl-IMPY by various IMPY derivatives. As shown in figure 3, *N*-succinyl-, *N*-formyl-, and *N*-acetyl-IMPY all showed greater affinities to the antibody than did IMPY. This is not surprising because *N*-succinyl-IMPY is the immunogen for raising the antibody, and the amide bond, which is absent in the parent drug, can be included as part of the antigenic determinant. Thus, the *N*-acylated analogs, which resemble the structure of the *N*-succinyl-IMPY with an amide side chain, would have better interactions with the antibody. The *N*-β-ribosyl-IMPY shows less cross-reactivity and the concentration required for 50% binding inhibition of [^{3}H]acetyl-IMPY is 40-fold higher than for IMPY.

TABLE 1.—Pharmacokinetic data

Patient No.	IMPY dose (mg/m^2/day)	Hepatic function	Day 1 Terminal half-life (hrs)	Day 1 Concentration × time (µg/ml·hr)	Day 1 V_d (ml/kg)	Day 5 Terminal half-life (hrs)	Day 5 Concentration × time (µg/ml·hr)	Day 5 V_d (ml/kg)
1	450	Minimally abnormal*	3.5	133	242	3.5	214	127
2	722	Normal	3.0	518	134		ND†	
3	1000	Minimally abnormal*	3.7	579	82	3.5	589	113
4	1000	Normal	4.8	1125	60	4.1	1046	111
5	1000	Minimally abnormal*	9.9	2203	119		ND†	
6	1500	Normal	5.1	1452	134	4.7	1393	147
7	1500	Normal	6.4	1853	105		ND†	

*Alkaline phosphatase: 146, 101, and 153 IU for patients 1, 3, and 5, respectively; LDH: 254 IU for patient 1; SGOT: 86, 81, and 71 mg/100 ml for patients 1, 3, and 5, respectively.

†Not determined.

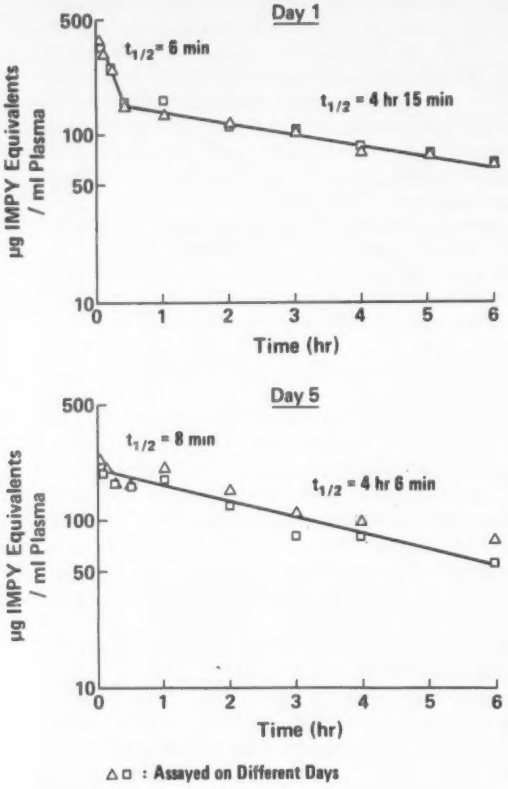

FIGURE 4.—Plasma drug disappearance curves in man. A dose of IMPY (1000 mg/m²/day) was iv infused in 30 mins. Immediately following the infusion, blood samples were collected and the drug contents were determined in duplicate by radioimmunoassay.

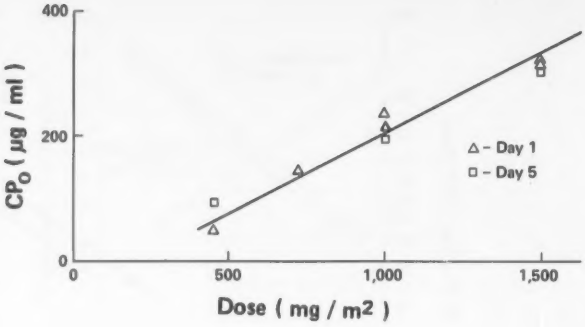

FIGURE 5.—Correlation of IMPY dose and initial plasma level (CP_o).

While this work was in progress, detection of IMPY metabolites from dog urine samples was reported (6). Those included the *N*-substituted derivatives. Although detailed metabolism of the drug in man is still unknown, the dog studies (6) and our preliminary results from gas chromatography indicate that the *N*-substituted metabolites are possible. These metabolites may cross-react with the antibody, and their interference with the radioimmunoassay can become significant when the concentration of IMPY is low. For these reasons, we have expressed our data in IMPY equivalents. The pharmacokinetics show that the disappearance of the drug in plasma is biphasic with a terminal half-life of 4 hours (fig 4). Staubus et al (4) reported an average half-life of 7.1 hours (range, 5.2–12) for six patients receiving a dose of 2700 mg/m²/day for 1 day. They suggested that phenotype differences in patients might be responsible for this large variation. The Day 1 and Day 5 pharmacokinetics were compared in four of our patients (two with minimal abnormal hepatic function). The data obtained were very similar, thus suggesting that there is no cumulative metabolic effect on IMPY pharmacology. Drug concentrations in intracerebral tumor were comparable to concurrent plasma concentrations suggesting that no barrier existed to its entry into this tumor. However, it must be noted that concentrations in intracerebral tumor were far lower than those in the temporalis muscle used as an extracerebral control. The brain tissue sampled was edematous and immediately adjacent to intracerebral tumor; hence, IMPY concentrations in it may not accurately reflect IMPY concentrations in normal human brain.

No definite antitumor activity of IMPY was demonstrated in the phase I trials, and further clinical study was prevented by its hemolysis toxicity (5). Brockman et al (3) suggested that a metabolite might be responsible for IMPY activity. This was supported by Neidhart et al (7) who demonstrated the [³H]thymidine uptake inhibition and cell synchronization of L1210 cells by β-ribosyl-IMPY. The possibility that an oxygen metabolite formed in vivo may be related to the hemolysis has also been mentioned (7). If the antitumor effect and toxicity are caused by two different derivatives, the IMPY therapeutic index could possibly be improved by en-

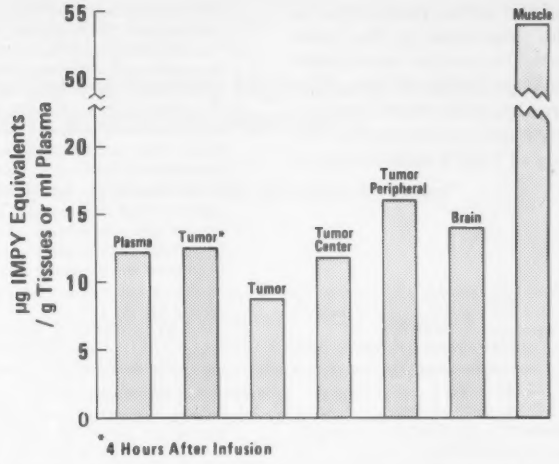

FIGURE 6.—Tissue levels in a patient with glioblastoma. 4–5 hrs after IMPY (500 mg/m^2) was iv infused into the patient, glioblastoma, brain, temporalis muscle, and plasma samples were obtained during surgery, and drug contents were determined by radioimmunoassay.

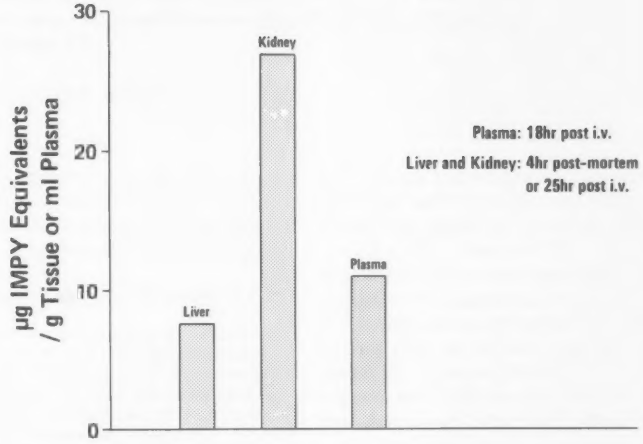

FIGURE 7.—Liver and kidney samples were obtained 4 hrs after death or 25 hrs after IMPY (1500 mg/m^2/day) for 3 days. Drug content was compared with a plasma sample which was drawn at 18 hrs after iv or 3 hrs before death.

hancing the formation of the active metabolite as well as by inhibiting the production of the toxic metabolites. Furthermore, the active metabolite could replace IMPY itself as a chemotherapeutic agent if production in large quantity were feasible. However, those approaches will not be possible until a better understanding of IMPY metabolism is achieved.

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Leukoencephalopathy Following High-Dose Iv Methotrexate Chemotherapy With Leucovorin Rescue[1,2,3]

Jeffrey C. Allen,* Gerald Rosen, Bipin M. Mehta, and Bruce Horten[4]

SUMMARY

Seven patients with bone or soft tissue sarcomas but without metastatic CNS disease developed a chronic leukoencephalopathy after high-dose (8000–15,000 mg/m²) iv methotrexate (MTX) chemotherapy with leucovorin (LV) rescue. Approximately 12 MTX-LV treatments were administered over a 3–7-month period. None of the patients had cranial irradiation. The syndrome usually began several months after the initiation of chemotherapy with subtle personality changes followed by a progressive dementia, focal seizures, pseudobulbar palsy, spastic quadriparesis, and stupor. Computerized tomographic scans revealed diffuse white matter hypodensity in five patients and atropic changes in five patients. Serum MTX concentrations were elevated in four of six patients prior to several MTX-LV treatments, suggesting that MTX persisted in tissues for a long time. Abnormally high levels of MTX were detected in the cerebrospinal fluid of all four patients several days after an MTX-LV treatment, at a time when their encephalopathy was most severe. Pathologic brain material was obtained from three patients and revealed a spectrum of abnormalities. The syndrome observed in our patients clinically resembles the one described in children with acute lymphatic leukemia who received cranial irradiation and large cumulative amounts of low-dose (12–20 mg/m²) systemic MTX without LV.

[Cancer Treat Rep 64:1261–1273, 1980]

Children with acute lymphocytic leukemia (ALL) may occasionally develop a methotrexate (MTX)-related leukoencephalopathy with signs of progressive ataxia, spasticity, and dementia (1). Affected individuals have usually received prior cranial irradiation and a large cumulative amount of relatively low doses of systemic MTX without leucovorin (LV) rescue (2). A clinically similar leukoencephalopathy has been observed in seven patients with sarcoma, who were treated with multiple courses of high-dose iv MTX followed by LV rescue and no cranial irradiation. Three of these cases have been alluded to elsewhere (3); in this report, we wish to describe in detail the clinical, radiologic, and neuropathologic manifestations.

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[3]Presented in part at the 8th Annual Meeting of the Child Neurology Society, Hanover, NH, Sept 13, 1979.

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CASE REPORTS

Patient 1

A 7½-year-old female with an osteosarcoma of the right femur had diffuse pulmonary metastatic lesions at the time of presentation. She was treated aggressively with chemotherapy and recurrent

wedge resections of the pulmonary metastatic lesions. Over a period of 7 months, she received 12 courses of MTX-LV with vincristine followed by 3 days of LV (10 mg orally every 6 hours for 3 days). The dose of MTX was initially 10 g/m² and was gradually escalated to 20 g/m² by the tenth treatment because of resistance and progression of disease.

The patient had one episode of systemic toxic reaction to the MTX chemotherapy (fever, rash, mucositis, and diarrhea) after the tenth course, but otherwise tolerated the chemotherapy well. In addition to the MTX, she received two courses of bleomycin, cyclophosphamide, actinomycin D, and doxorubicin (T-7 protocol) (4). Her disease was never adequately controlled by the chemotherapy.

The earliest signs of clinical encephalopathy were difficult to document. Several months after the initiation of chemotherapy, the parents noted a subtle change in the child's personality. Initially she was observed to be apathetic, withdrawn, and possibly depressed, but by the sixth month she became easily confused and lost the ability to feed and dress herself. She became progressively clumsy and had difficulty walking with her crutches. By the seventh month she was stuporous, demented, incontinent of urine and feces, and had a severe spastic quadriparesis with a pseudobulbar palsy. She became comatose and manifested decorticate posturing and diffuse pathologic reflexes.

Serum was assayed for MTX by a microbiologic assay (5) prior to each treatment. Measurable MTX ($1–5 \times 10^{-8}$ M) was found in five of 12 assays (prior to the administration of the seventh, eighth, ninth, tenth, and 12th courses of MTX), indicating a failure to completely eliminate the previously administered drug. After the 12th MTX-LV treatment, when her encephalopathy was most evident, cerebrospinal fluid (CSF) MTX values 3 and 7 days after treatment remained elevated at 2.5×10^{-8} and 0.4×10^{-8} M, respectively, and serum MTX was 5.6×10^{-8} M at 7 days.[5]

The EEG after the 12th treatment showed diffuse delta slowing, and the computerized tomographic (CT) scan, with and without contrast enhancement, revealed diffuse hypodensity of cerebral white matter, especially anterior to the frontal horns. A second CT scan 1 month later indicated progressive enlargement of the lateral ventricles.

Although she improved neurologically 1 month after chemotherapy was discontinued, the child died from metastatic cancer 2 months later. An autopsy was not performed.

[5]Following a single course of MTX-LV, MTX is normally undetectable in the CSF by 48 hours (6).

Patient 2

An osteosarcoma of the distal left femur was discovered in a 6-year-old white male. There was no evidence of metastatic spread at the time of diagnosis. The T-7 protocol was followed. He received a total of 12 high-dose MTX treatments over a period of 5 months. The dose of MTX was initially 12 g/m² and was gradually increased to 18.7 mg/m² by the 11th treatment. There was little systemic toxicity from the MTX.

Early in the course of his chemotherapy, the patient complained of severe headaches occurring several days after high-dose MTX chemotherapy. These lasted for several days and seemed to recur in a similar pattern after each MTX treatment. After his seventh MTX treatment, personality changes were observed. His behavior vacillated from irritability to lethargy. These symptoms gradually cleared over 2 weeks' time.

After the 12th course of chemotherapy, progressive deterioration of the patient's neurologic function was noted. He initially began to drool uncontrollably and developed slurred speech, clumsiness, and ataxia. He subsequently lapsed into a semicomatose state and had continual high fevers of unexplained etiology. He remained stuporous for several months and developed extreme trismus, neck stiffness, and decerebrate posturing. He drooled constantly and had urinary and fecal incontinence. High doses of iv LV were administered with no apparent effect.

The patient gradually improved over several months. His language abilities returned slowly and he was able to swallow semisolid foods. Ten months after stopping MTX therapy, he continues to improve to the point where he now is able to feed himself, express simple needs, and acquire a few self-help skills; however, he retains mild dementia, spastic quadriparesis, and pseudobulbar palsy. He remains free of osteosarcoma.

An EEG during the time of the most severe stage of encephalopathy showed diffuse slowing with intermittent multifocal epileptiform discharges, and a CT scan indicated disseminated white matter hypodensity. Areas of the contrast enhancement were apparent anterior to the frontal horns. Subsequent CT scans over the following 11 months showed slight improvement in the white matter hypodensity, but the ventricles and cortical sulci progressively enlarged. The patient did not have any serious systemic toxic reactions after any of the MTX treatments.

Patient 3

This case has been previously reported (7). A 14-

year-old white male was referred to Memorial Sloan-Kettering Cancer Center with progressive pain and swelling of the right ankle and massive multiple pulmonary metastases from the primary lesion, thought to be a chondroblastoma. Chemotherapy was instituted with MTX-LV (8 g/m^2) and vincristine, and the dose of MTX was gradually escalated to 20 g/m^2 over 5 months in an attempt to obtain a clinical regression of disease. He received 12 MTX-LV treatments over 5 months.

Fourteen weeks after the initiation of chemotherapy, by the ninth course of MTX-LV, the child became lethargic, disinterested, and confused. He complained of episodic numbness in his left hand. On one occasion, these paresthesias were accompanied by a brief loss of consciousness followed by a transient dysarthria and left hemiparesis. Subsequently, he was treated with Dilantin for a presumed idiopathic focal seizure disorder. A CT scan performed 16 weeks after the initiation of MTX-LV chemotherapy revealed a mild hypodensity of the centrum semiovale (fig 1, left).

By the 20th week, the patient became progressively encephalopathic with incontinence, spastic quadriparesis, and pseudobulbar palsy. He began to experience intermittent right-sided paresthesias, developed a profound dementia, and ultimately lapsed into stupor.

At the ninth MTX-LV treatment, the pretreatment serum assay showed persistent low levels of MTX in the range of 1–5 $\times$ 10^{-8} M. The EEG was diffusely slow, and a CT scan at 20 weeks (fig 1, center) revealed a further decrease in white matter attenuation. At lumbar puncture, the opening pressure was normal, 3 mononuclear cells/mm^3 were observed, the protein was 38 mg/100 ml, and the sugar was 63 mg/100 ml.

Consequently, MTX therapy was withheld. Large doses of iv LV were administered without apparent benefit. The patient gradually improved over the next 12 months while receiving different chemotherapy, and he remains moderately demented, dysarthric, and mildly quadriparetic. Psychometric testing (WISC) performed 12 months after the initiation of MTX-LV therapy indicated an IQ of 75, and a contemporaneous CT scan revealed hydrocephalus (fig 1, right). The patient's tumor response to chemotherapy has been sustained approximately 30 months after he presented with terminal pulmonary metastatic disease.

Patient 4

A 21-year-old black female developed a swelling of the left thigh and a biopsy revealed a low-grade fibrosarcoma. A left hemipelvectomy was performed, but 2 months later the tumor locally recurred. The patient received 12 MTX-LV treatments over 15 weeks. The initial four doses were in the range of 9.7 g/m^2, but because of failure of control of local disease, the dose was gradually escalated to 12.4 g/m^2 by the sixth treatment.

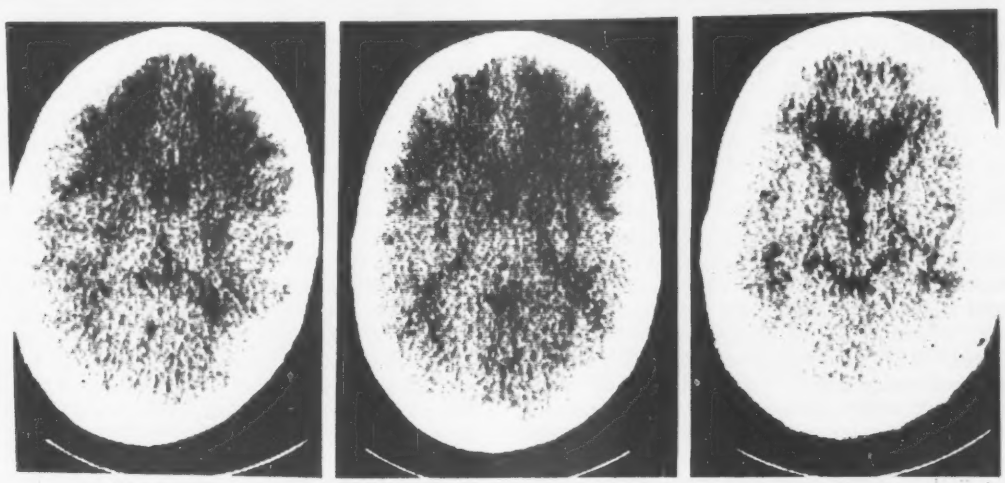


FIGURE 1.—CT scans from Patient 3. 16 wks after the initiation of high-dose MTX chemotherapy, a mild hypodensity of the centrum semiovale is apparent (*left*). The 20-wk scan (*center*) showed further progression of white matter hypodensity. The 12-mo scan (*right*) demonstrates hydrocephalus presumably related to diffuse cerebral atrophy and persistent white matter hypodensity.

TABLE 1.—Serum + CSF MTX when encephalopathy is severe

Patient No.	High-dose MTX treatment	No. of days after high-dose MTX	MTX concentration ($\times 10^{-8}$ M)	
			Serum	CSF
1	12th	3	3.4	2.5*
		7	5.6	0.4
3	12th	6	2.7	1.9
		11	2.0	NMA†
4	12th	4	3.7	8.4
5	7th	2	160	90
		9	9.3	1.9

*MTX usually cleared from CSF by 48 hrs.
†NMA = no measurable amount.

The chemotherapy was well-tolerated systemically and the pretreatment serum MTX levels were negative at all times. Approximately 10 weeks after the initiation of chemotherapy, the patient's behavior changed. She became relatively apathetic and had periods of confusion and disorientation.

After her 12th MTX-LV treatment she had a mild systemic toxic reaction with fever, mucositis, and dehydration. During physical examination she appeared withdrawn, apathetic, and bradykinetic. She had a mild dementia and diffuse pathologic neurologic reflexes with paratonia. A CT scan with and without contrast was normal. Her serum MTX concentrations were within the nontoxic range but a CSF MTX level measured 4 days after the 12th treatment was more than twice that of serum (table 1). An EEG showed diffuse delta slowing.

The patient was readmitted approximately 3 weeks later for a right cerebral focal seizure disorder. Since discharge, she had become progressively more stuporous and had lost all interest or ability to verbally communicate. Although awake, she failed to follow simple commands or attend to her personal needs. She was incontinent of urine and feces and was incapable of eating or drinking. She had intermittent left-sided focal seizures.

In addition to diffuse pathologic reflexes, pseudobulbar palsy, spastic quadraparesis, and bilateral Babinski signs were revealed by examination. An EEG showed an epileptiform abnormality in the right frontal temporal region with diffuse delta slowing. A CT scan indicated a discrete 1-cm contrast-enhancing lesion in the right frontal region (fig 2, left) with a diffuse hypodensity of cerebral white matter in the right hemisphere (fig 2, right). A cerebral angiogram indicated an avascular mass in the right frontal region. The opening pressure was normal at lumbar puncture; 1 mononuclear cell/mm^3 was observed, the protein was 47 mg/100 ml, and the sugar was 52 mg/100 ml.

A needle biopsy of the right frontal lesion was performed (fig 3). On microscopic examination, the cortex was unremarkable. However, focally there was a coagulative necrosis within white matter with a fibrinoid necrosis of small vessels, probably arterioles and perivascular dystrophic calcification. The surrounding brain revealed focal axonal swellings, pallor of the neuropil, and clusters of lipid-laden macrophages, most abundant in a perivascular distribution. The histologic pattern was compatible with an MTX-induced leukoencephalopathy (8).

The patient was nourished through a gastrostomy feeding tube and discharged after 1 month with slight neurologic improvement. The patient died of progressive local malignancy 11 months after beginning MTX-LV treatment. At autopsy the brain was diffusely atrophic (1000 g) with hydrocephalus ex vacuo and scattered white matter cavitation most marked in the right frontal lobe. Microscopic examination confirmed the biopsy impression of MTX leukoencephalopathy with congruent foci of white matter necrosis characterized by myelin loss, clusters of macrophages, and dystrophic axons with mineral deposition.

Patient 5

A 23-year-old white male presented with an osteosarcoma of the left femur 5 years ago in another hospital. A mid-thigh amputation was performed. Three years later, pulmonary metastatic disease was discovered and the right lower lobe was resected. For 1 year, he received chemotherapy consisting of doxorubicin (60 mg/m^2) and MTX-LV (2.1 g/m^2), given every 6 weeks.

The patient's disease continued to progress and he was referred to Memorial Sloan-Kettering Cancer Center with metastatic lesions in both lung fields and in the proximal right femur. There, he

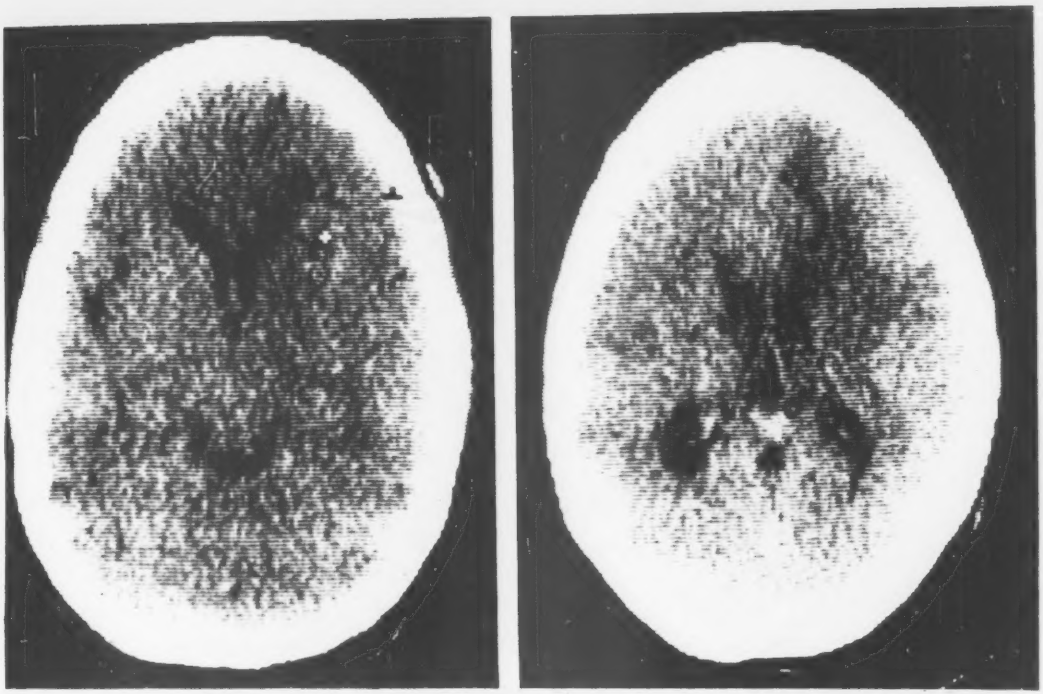


FIGURE 2.—CT scans from patient 4. The CT scan obtained 15 wks after the initiation of high-dose MTX chemotherapy (*left*) indicates a discrete, 1-cm, contrast-enhancing lesion in the right frontal region. + = area biopsied. A higher cut (*right*) indicates extensive hypodensity of surrounding white matter of the right hemisphere.

received seven courses of MTX-LV (8.0 g/m²) over a period of 8 weeks. He developed signs of systemic toxicity (mucositis, fever, leukopenia, and thrombocytopenia) on three occasions: after his second, fifth, and seventh treatments.

One week prior to the seventh MTX-LV treatment he became mildly confused, had hallucinations, and acquired inappropriate behavior. Twenty-four hours after his seventh treatment he was observed to be disoriented, confused, and hallucinatory with a mild dementia. He was preoccupied with picking at his clothes and climbing out of his bed despite restraints. He had diffuse pathologic reflexes including an active suck and snout reflex, bilateral grasp reflexes, and hyperactive jaw jerk.

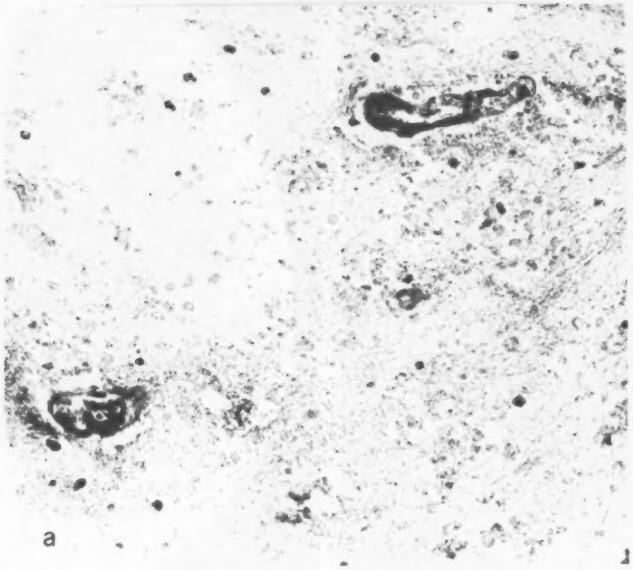
The 24-hour serum MTX value was high and he continued to have elevated serum MTX for 12 days despite daily oral administration of LV (10 mg) every 6 hours and iv hydration for 2 weeks.

He also had measurable MTX in CSF at 57 hours and at 9 days (table 1). The patient gradually improved over the next 6 weeks but retained a diffuse encephalopathy with intermittent confusion and a mild dementia. An en bloc resection of the metastatic lesion in his right femur was performed and the pulmonary metastases were resected. No viable tumor was encountered on pathologic examination.

A CT scan performed at the time of his seventh treatment was normal; a repeat scan done 3 weeks later showed normal cerebral white matter but mild ventricular enlargement presumably related to cerebral atrophy. An EEG showed diffuse bilateral delta slowing. The pretreatment serum MTX value was elevated on only one occasion, but the 24-hour MTX level was > 5000 ng/ml at four of the seven MTX-LV treatments. This 24-hour value has been associated with systemic toxicity.

Patient 6

A 15-year-old black male complained of increasing right arm and shoulder pain and weakness for 6 months prior to hospital admission. He also noticed enlargement of his right shoulder. Biopsy of the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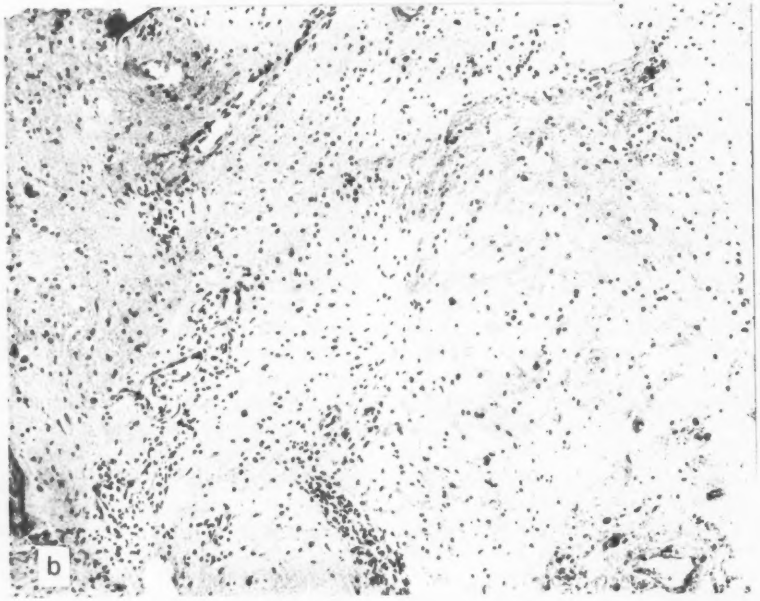
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FIGURE 3.—Needle biopsy of the right frontal lobe of patient 4 (*see* fig 2). (*a*) Fibrinoid necrosis is apparent within small vessels, probably arterioles with surrounding coagulative necrosis. PTAH stain, × 250. (*b*) There is evidence of coagulative necrosis of white matter towards the right, with adjacent reactive tissue. H and E stain, × 100. (*c*) In addition to coagulative necrosis of white matter towards the right, an axonal stain reveals evidence of axonal swelling and there is evidence of adjacent reactive tissue as well. Bielschowsky stain, × 250.

mass indicated a chondrosarcoma which involved the apical region of the right hemithorax invading the brachial plexus. There was no evidence of metastatic disease. The patient was treated with 11 courses of MTX-LV therapy over a 3-month period. The initial dose was 7.6 g/m². Because of lack of response, the dose was escalated by the fourth treatment to 17.6 g/m².

After the sixth MTX-LV treatment the patient noted severe frontal headaches and photophobia. His family had observed a change in his behavior and intellect. He had become increasingly apathetic and lethargic and evidenced intermittent confusion, disorientation, and mild dementia. He acquired diffuse pathologic neurologic signs such as snout, suck, and bilateral grasp reflexes.

A CT scan with and without contrast was normal, but the EEG was markedly abnormal with diffuse slowing in the delta frequency. At lumbar puncture he had an opening pressure of 140 mm of H_2O, a wbc count of 39/mm³ (89% leukocytes), a protein of 73 mg/100 ml, and a glucose of 76 mg/100 ml. A WISC psychometric test was performed and the patient functioned in the dull normal range with a full-scale IQ of 86. The pretreatment serum MTX values were elevated on at least four occasions. The patient had two episodes of systemic toxic reactions (mucositis, fever, and diarrhea) requiring hospitalization.

The patient improved gradually but never fully recovered his prior intellectual functions. He died of uncontrolled intrathoracic disease 5 months later. He was mildly hypoxic for 2 days prior to death. At autopsy, there were no gross abnormalities of the brain, but microscopic examination revealed a diffuse spongy state within cerebral white matter with associated astrocytosis (fig 4). The remainder of the microscopic examination was unremarkable.

Patient 7

A 30-year-old white male presented with an osteosarcoma of the left pelvis. There was no evidence of metastatic spread at the time of diagnosis. The patient was treated with eight courses of MTX-LV over a period of 4 months. The initial dose was 7.5 g/m². Because of failure of response, the dose was gradually escalated to 11 g/m². After the eighth MTX-LV treatment, he received combination chemotherapy with bleomycin, cyclophosphamide, actinomycin D, and subsequently cisplatin.

The ninth and final high-dose MTX therapy was administered 1 month after the eighth MTX-LV treatment. Approximately 4 days later, the patient showed a change in personality. He became intermittently confused and hallucinatory and developed a memory impairment. Within several days he acquired mild left hemiparesis and left central facial weakness. He also had diffuse pathologic reflexes

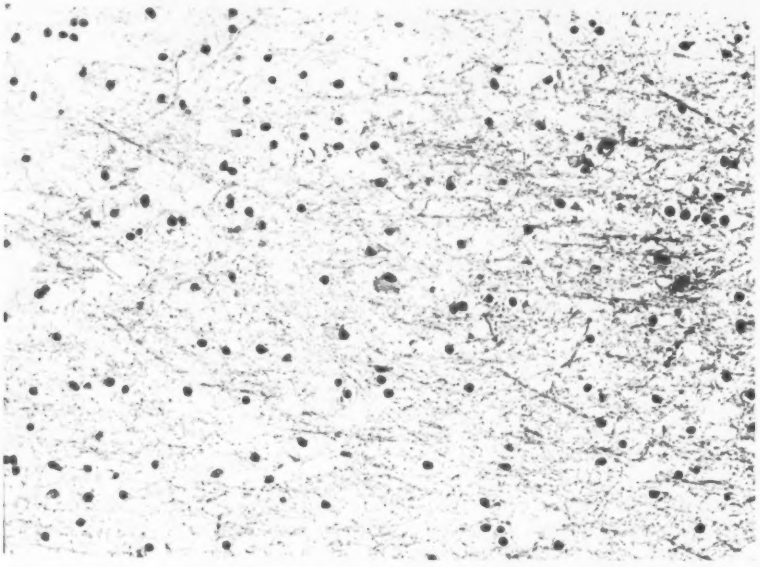

FIGURE 4.—Autopsy material from subcortical white matter of patient 6. There is evidence of diffuse spongy change and rare reactive astrocytes (*center of field*). H and E stain, × 250.

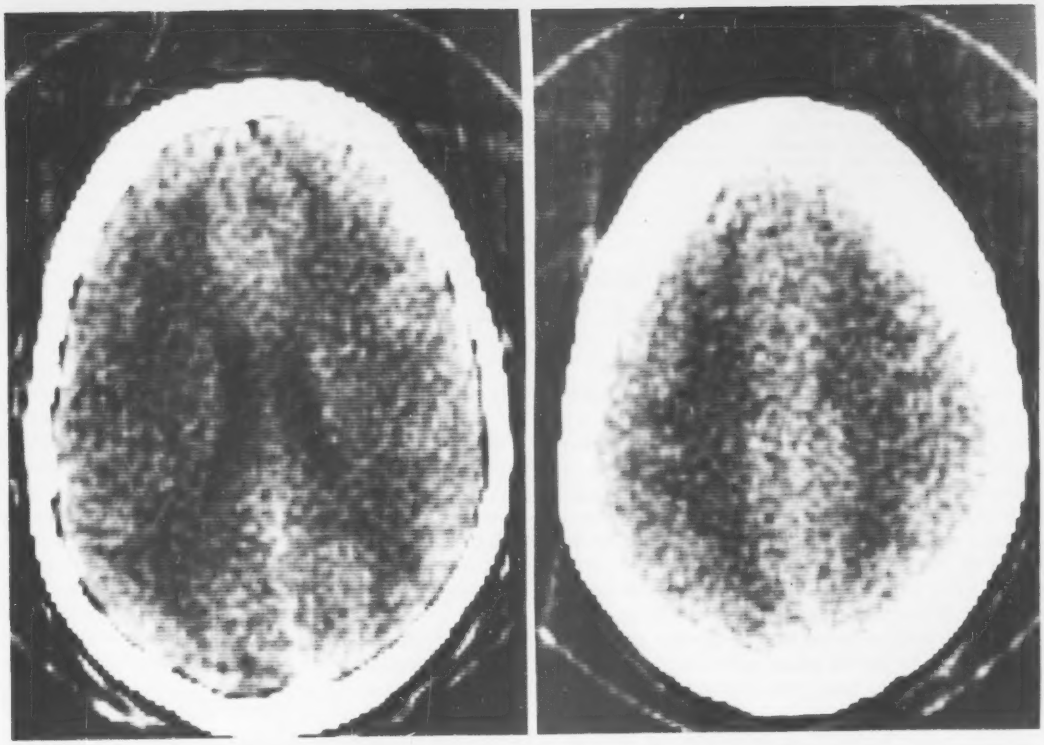

FIGURE 5.—CT scan from patient 7. The CT scan was obtained 5 mos after the initiation of high-dose MTX chemotherapy. There is evidence of mild ventricular enlargement and periventricular white matter hypodensity (*left*). The hypodensity is apparent throughout cerebral white matter in cuts above the lateral ventricles (*right*).

such as bilateral grasp and snout reflexes, and diffuse paratonia.

The CT scan at this time indicated diffuse periventricular white matter hypodensity and mild ventricular enlargement (fig 5). At lumbar puncture his opening pressure was normal; protein was 16 mg/100 ml and glucose was 50 mg/100 ml.

No MTX was detectable in the serum prior to any of the nine MTX-LV treatments. He had not had any excessive systemic toxic reactions to MTX. The patient improved minimally in terms of his mental status and died 1 month later from his cancer. He was mildly hypoxic for 48 hours prior to his death.

At autopsy, the brain was grossly unremarkable but microscopically showed a diffuse spongy state within white matter, especially prominent in periventricular and subcortical sites, closely resembling that described for patient 6 (fig 6). This spongy change was accompanied by a diffuse mild fibrillary astrocytosis (fig 6a) and by clusters of plump, lipid-laden macrophages (fig 6b). Special stains for myelin (LFB, PAS) and axons (Bielschowsky) revealed nothing remarkable, nor did the remainder of the microscopic examination.

DISCUSSION

Seven patients with sarcoma acquired chronic encephalopathy after receiving multiple courses of iv MTX-LV for 2–7 months (table 2). Their initial symptoms began insidiously, usually with subtle personality changes within 1–5 months after the initiation of chemotherapy (table 3).

Although the syndrome eventually tended to manifest itself in a global and symmetrical fashion, three patients (Nos. 3, 4, and 7) had focal neurologic disturbances early in their course. Patient 3 presented with focal seizures but eventually developed a diffuse encephalopathy. Patient 4 also had focal seizures and the initial diagnostic studies pointed to an avascular lesion in the right frontal lobe. However, she too subsequently developed diffuse en-

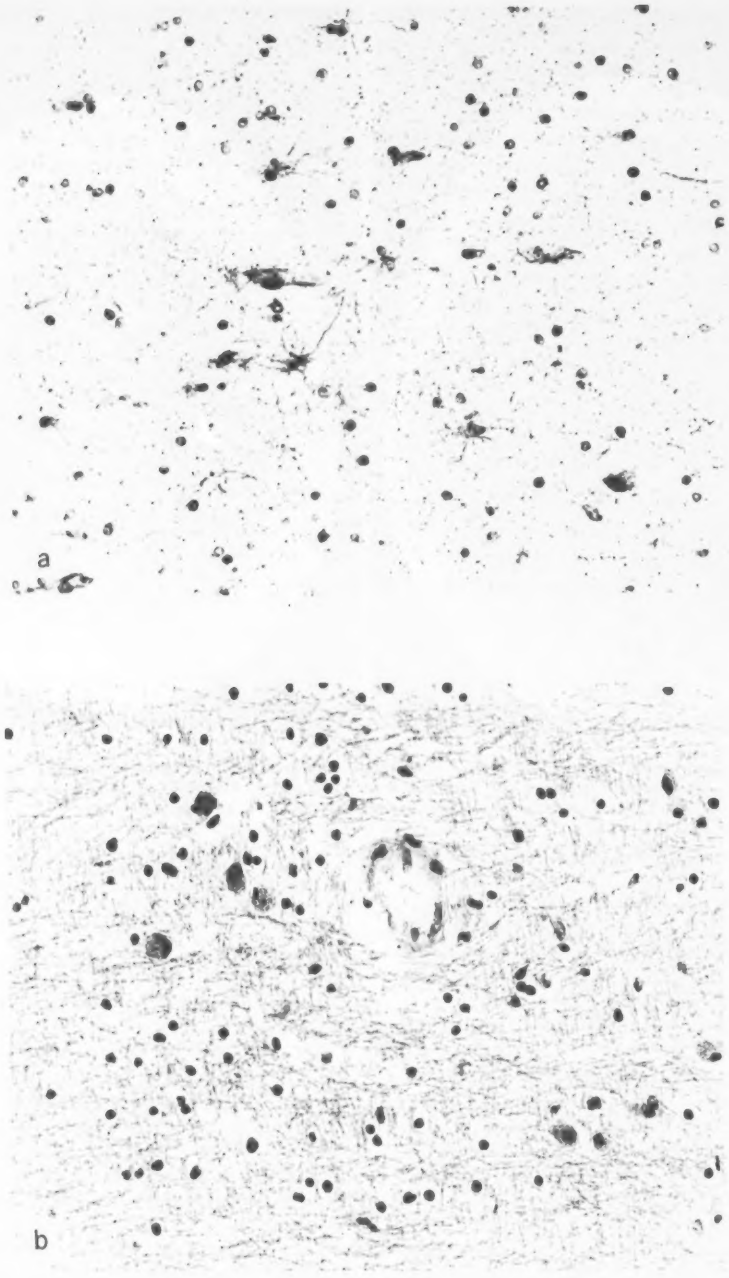

FIGURE 6.—Post-mortem examination of subcortical white matter from patient 7. (*a*) Scattered reactive astrocytes are apparent throughout subcortical white matter. Holzer stain, × 250. (*b*) The periventricular white matter has clusters of lipid-laden macrophages. H and E stain, × 250.

TABLE 2.—Clinical information

Patient No.	Tumor/stage	Age (yrs)	Sex	No. of treatments, interval	Dose range (g/m^2)	Systemic MTX toxicity (No. of episodes)	CNS MTX toxicity	
							Onset after beginning high-dose MTX (mo)	Peak severity of encephalopathy (mo)
1	Osteosarcoma/IV	7½	F	12, 7 mos	10–20	1	2nd	7th
2	Osteosarcoma/I	6	M	12, 5 mos	12–19	—	1st	5th
3	Chondroblastoma/IV	14	M	12, 5 mos	8–20	—	3rd	5th
4	Fibrosarcoma/I	21	F	12, 4 mos	9.7–12.4	1	3rd	12th
5	Osteosarcoma/IV	23	M	7, 2 mos	8	3	2nd	2nd
6	Chondrosarcoma/III	16	M	11, 3 mos	7.6–17.6	2	3rd	3rd
7	Osteosarcoma/IV	30	M	9, 5 mos	7.5–11	—	5th	5th

cephalopathy. Patient 7 had mild left hemiparesis at the time of diagnosis.

After several months these patients acquired progressive neurologic deficits affecting mentation, motor power, tone and coordination, and control of eating and drinking, speech, and sphincters. Alterations in level of consciousness ranged from frank stupor (patients 1–3) to delirium (patients 5 and 7). Two patients (Nos. 4 and 6) became withdrawn and apathetic.

In all seven patients, the syndrome affected the quality of survival. Each patient retained varying degrees of neurologic deficits, although most patients improved after MTX-LV chemotherapy was discontinued. Patient 1 died with progressive systemic tumor and improved slightly over a 2-month period to where she was able to converse in full sentences and feed and dress herself, but she died of untreated, progressive osteosarcoma. The child who remains osteosarcoma-free (patient 2) retains mild dementia, and is incapacitated by pseudobulbar palsy and spastic quadriparesis. He drools continually, but appears to be improving slowly.

Patient 3 has residual metastatic disease and is being treated with alternative chemotherapy. He also retains mild dementia, pseudobulbar palsy with profound dysarthria, and uncontrollable drooling. However, he is ambulatory and has re-acquired self-help skills, such as feeding and dressing. Patient 4 retained profound dementia and all chemotherapy had been discontinued prior to her death. Patient 5 has only mild dementia but has intermittent periods of confusion and inappropriate behavior. His metastatic tumor has responded completely to the MTX-LV and no further chemotherapy is being considered. Patient 6 refused further chemotherapy after developing mild dementia. He died of his cancer 6 months later. Patient 7 died within 6 weeks of acquiring encephalopathy, and only minimal neurologic improvement was observed.

The CT scan was very useful diagnostically (table 4) in that the majority of patients had CT scans similar to those described in patients with ALL who had documented leukoencephalopathy with diffuse white matter hypodensity (9,10). The white matter anterior to the frontal horns was usually the first area to appear hypodense, but eventually most cerebral white matter was affected. Four of seven early scans and four of five late scans had this appearance. Evidence of sulcal and ventricular enlargement was apparent either at the time of diagnosis or in followup scans in five of seven cases, which was consistent with loss of cerebral parenchyma. No intracerebral calcifications were detected on CT scanning.

This chronic leukoencephalopathy should be distinguished from another acute and transient encephalopathy affecting osteosarcoma patients receiving MTX-LV, where multifocal cerebral disturbances were observed earlier in the course of chemotherapy (11). In the latter syndrome, dementia was not detected and most of the patients regained a normal neurologic status despite the continuation of MTX-LV chemotherapy.

The syndrome described in this report resembles the chronic progressive leukoencephalopathy which occasionally affects children with ALL who have been treated with cranial irradiation and systemic and/or intrathecal MTX without LV (1).

This syndrome usually begins insidiously with behavioral changes, followed by increasing confusion and decreasing motoric and psychologic activity. In its severe form, profound dementia, spasticity, and stupor supervene (1). It is likely that formes frustes of this syndrome exist in a larger group of ALL patients. The syndrome may evolve over 3–6 months, usually after the first year of chemotherapy, and is pathologically characterized by the presence of discrete multifocal areas of coagulative necrosis of cerebral white matter (2,8).

TABLE 3.—Clinical course in 7 patients with encephalopathy

Initial symptoms			Late symptoms		
Personality change	Focal seizures	Focal signs	Dementia	Quadriparesis	Stupor
7	2	3	7	7	4

TABLE 4.—CT scans in patients with encephalopathy

Observation	No. of patients	
	Early scans* (7 patients)	Late scans† (5 patients)
Normal	3	0
Diffuse white matter hypodensity	4	4
Cerebral atrophy	1	4
Focal lesion	0	1

*At time of diagnosis of encephalopathy.
†3–12 mos after diagnosis.

The etiology of the syndrome in ALL is unknown but may be related to chronic depletion of CNS folates. Factors which may increase the risk for acquiring MTX-related leukoencephalopathy in the leukemic population include, in addition to cranial irradiation, hydrocephalus, and CSF stasis, the presence of meningeal leukemia and large cumulative doses of iv methotrexate (12–14). One case has been observed in a child with ALL receiving only systemic and intrathecal MTX without cranial radiation therapy (15).

For ALL, MTX is usually given in relatively low iv or intrathecal doses (10–20 mg/m²) without LV rescue. Massive doses of iv MTX (8–15 g/m²) with LV have proved effective in controlling metastatic osteosarcoma and producing long-term disease-free survival in osteosarcoma patients receiving adjuvant chemotherapy (3). The CNS is perfused with MTX during MTX-LV treatment, and MTX may be detectable in the CSF for up to 48 hours (6). Osteosarcoma patients, however, were considered to be relatively immune to the CNS toxicity of MTX therapy.

The etiology of the leukoencephalopathy syndrome in our sarcoma patients is equally uncertain. Vincristine could not seriously be implicated since only three of seven patients received concomitant vincristine. The patients were treated more aggressively with MTX-LV than the average osteosarcoma patient in our institution because of their relatively poor prognosis. Persistent low levels of pretreatment serum MTX were detected on several occasions in four of six patients, suggesting that their bodies were saturated with MTX for an extended period of time. Systemic MTX toxicity occurred after < 10% of the treatments (seven of 75; table 2). Abnormally high levels of MTX were detected in the CSF in all four patients so investigated (Nos. 1, 3, 4, and 5) several days after an MTX-LV treatment, at a time when encephalopathy was most severe. No CSF specimens were obtained from patients 2, 6, and 7 (table 1).

The MTX may have remained in low concentration over a long period of time within the CNS because of the relatively short interval between MTX-LV treatments and the relatively high doses of MTX employed. Although oral LV may effectively rescue folate-depleted cells outside the CNS, the levels of citrovorum factor achieved in the CNS are probably inadequate to rescue folate-depleted enzyme systems (16). MTX rescue in the CNS, therefore, primarily depends on clearance of MTX from the body by renal excretion.

MTX is thought to exert its principal effect on cancer cells by inhibiting the enzyme dihydrofolate reductase and the subsequent formation of tetrahydrofolate which is essential for methylation reactions in pyrimidine and purine synthesis involved in the formation of DNA. However, there is a relatively low rate of DNA synthesis in slowly or nondividing cells within the CNS. More likely, MTX may inhibit folate-requiring enzyme reactions in other metabolic pathways required for neurotransmitter and protein synthesis (17). Why cerebral myelin appears the most neuropathologically sensitive to MTX is unknown.

Neuropathologic examination of the biopsy specimen from patient 4 and in the autopsies of patients 6 and 7 reveals a spectrum of white matter lesions ranging from mild spongy change with astrocytosis (patient 6) to coagulative necrosis, axonal swelling, angionecrosis, and dystrophic calcification (patient 4), with a possibly intermediate stage of extensive spongy change, astrocytosis, and focal macrophage formation (patient 7). Taken together, these cases suggest that the earliest morphologic expression of MTX leukoencephalopathy may be myelin swelling, followed by myelin degradation with macrophage formation and still later by axonal swelling and coagulative necrosis, and accompanied at all stages by reactive astrocytosis. The absence of grey matter

pathology suggests that terminal hypoxia, present in patients 6 and 7, did not cause the abnormalities observed in white matter.

In conclusion, it appears that patients receiving MTX-LV are at risk for developing leukoencephalopathy, despite the fact that they have not received cranial irradiation or intrathecal MTX. The incidence of the severe manifestations of this syndrome is fortunately low in our institution. Only seven well-documented cases have been observed in over 300 patients treated in the past 5 years.

However, a number of other patients may have had neurologic difficulties such as somnolence, personality changes, headaches, and seizures reflecting formes frustes of the leukoencephalopathy. Those patients at high risk appear to be those receiving very large doses of MTX (> 10 g/m²) at 7–10-day intervals. Preliminary observations suggest that high doses may be administered safely if the interval between treatments is at least 2 weeks. Prospective monitoring of pretreatment serum and CSF MTX and 5-methyl tetrahydrofolate levels, the degree of white matter hypodensity on CT scans, psychometric performance, and important changes in behavior and neurologic exam may allow the clinician to recognize early neurotoxic effects in patients at risk for developing this syndrome. Since many of the symptoms appear to be partially reversible, early detection may prevent the overt and permanent manifestations of this devastating syndrome. If signs of neurotoxicity arise, modifications of dose and lengthening of treatment intervals should be considered. In some cases, discontinuation of MTX-LV therapy would be indicated.

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Variability in DNA Distributions of Human Neuroblastomas After Cyclophosphamide[1,2]

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SUMMARY

Major changes in DNA distributions of two human neuroblastoma cell lines growing in vitro and in athymic nude mice occurred after treatment with cyclophosphamide. Pulse treatment of LA-N-1 cells in vitro with liver S-9-activated cyclophosphamide (10 μg/ml) caused approximately 50% cytotoxicity; flow microfluorometric analysis of surviving cells demonstrated an increased proportion of G_2 + M cells and a decreased proportion of G_1 cells, particularly at 48 hrs. Even though LA-N-1 tumors in nude mice did not regress after one dose of cyclophosphamide (250 mg/kg), the percent of G_2 + M cells increased and the percent of G_1 cells decreased 4–6 days after treatment; the percent of cells in S increased at 2 and again at 8 days. SK-N-MC cells were affected differently by cyclophosphamide. Pulse treatment of these cells in vitro with liver S-9-activated cyclophosphamide caused > 85% cytotoxicity and nearly complete elimination of cells in G_2 + M at 24 and 48 hrs. Likewise, SK-N-MC tumors in nude mice regressed > 50% after cyclophosphamide, and the proportion of G_2 + M cells decreased markedly 2–6 days after therapy. We conclude that cyclophosphamide can have different cytotoxic and cytokinetic effects on neuroblastomas. In addition, marked cytokinetic effects may occur even though changes in tumor size are minimal or not detectable.

[Cancer Treat Rep 64:1275–1281, 1980]

The prognosis for most children with metastatic neuroblastoma has been poor (1,2), and even new therapeutic regimens being investigated do not appear to improve survival (3,4). More effective treatment must be developed, and this may be facilitated if cell kinetics are considered in the design of new regimens (5). Cellular DNA content can be used to characterize tumors with regard to the cell cycle; and, after appropriate staining, DNA can be measured rapidly with automated flow microfluorometry (FMF) (6–8).

Although cyclophosphamide is one of the most effective drugs for neuroblastoma (2), clinical re-

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sponsiveness varies; there are also data suggesting variability in cell kinetic responses (5). We previously demonstrated that LA-N-1 and SK-N-MC human neuroblastoma cells growing in vitro or in athymic nude mice differ in their sensitivity to cytotoxic effects of cyclophosphamide (9). In the present investigation, we found that cyclophosphamide induces readily detectable perturbations in DNA distributions that are different for the two cell lines.

MATERIALS AND METHODS

Growth of Neuroblastoma Cells and Treatment With Cyclophosphamide

Details of the two human neuroblastoma cell lines LA-N-1 (10) and SK-N-MC (11) and treatment of these cell lines with cyclophosphamide in vitro and in athymic nude mice (9,12) have been reported. For FMF studies of cells treated in vitro, 10^6 cells were removed from culture flasks by brief exposure to trypsin-EDTA (Flow Laboratories, Rockville, MD), incubated with Aroclor-induced liver S-9 and cyclophosphamide or with liver S-9 alone (37°C for 1 hr) (9), washed, plated into 60-mm diameter dishes, cultured, and analyzed after 9, 24, 48, 72, and 96 hrs. For in vivo experiments, mice bearing 10-mm diameter tumors were given one injection of cyclophosphamide (250 mg/kg ip), and were killed 2–10 days later for analysis of tumors.

Preparation of Neuroblastoma Cells for FMF Analysis

Culture dishes were gently washed once with Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS) to remove nonadherent dead and damaged cells, and then adherent cells were detached with trypsin-EDTA, dispersed into single-cell suspensions, and washed once with MEM-FCS. These cells were > 90% viable as assessed with 0.2% trypan blue in normal saline. Tumors from nude mice were minced into 1-mm^3 fragments and stirred in a trypsinizing flask with trypsin-EDTA. Single cells in the supernatant fluid were removed at 15-min intervals and placed in MEM-FCS, and fresh trypsin-EDTA was added to the remaining fragments. After repeating this three times, cells from the supernatant fluids were pooled, centrifuged, and resuspended in trypsin-EDTA at 37°C for 2 minutes, and were then filtered through 0.4 ml of loosely packed boiled cotton wool in a 5-ml syringe to remove nonviable cells. Those cells passing through the filter were > 90% viable as determined with trypan blue.

To stain DNA, 10^6 cells were pelleted, resuspended in 2 ml of 0.1% citric acid containing 15 μg/ml of propidium iodide (8), and incubated for 15 mins on ice. Cells were passed through a 40-μ mesh filter to remove any debris and clumps of cells immediately before FMF analysis. Comparative experiments demonstrated that citric acid-propidium iodide gave DNA distributions similar to those obtained with Feulgen (13), ethanol-mithramycin (6), RNAse-ethanol-propidium iodide (8,14), and RNAse-glutaraldehyde-propidium iodide staining procedures.

Cells were viably frozen before FMF analysis because this provided convenient storage and also allowed for analysis of multiple samples the same day under identical conditions. Single cells (2–5 × 10^6/ml) were suspended in MEM-FCS containing 10% dimethyl sulfoxide and viably frozen and stored in the vapor phase of liquid nitrogen; they generally were used within 1 month of preparation. When cells were to be analyzed, they were thawed rapidly by swirling vials in a 37°C water bath, diluted tenfold with MEM containing 10% FCS, centrifuged (250 × *g* for 10 mins), washed once with MEM-FCS, and then stained with citric acid-propidium iodide. In three experiments, the DNA distributions of both cultured cells and cells obtained from tumors in mice were the same before and after viable freezing. For example, a LA-N-1 tumor contained the following percent (mean ± 1 SD) of cells in G_1, S, and G_2 + M: fresh cells, 72 ± 1, 17 ± 1, 11 ± 1; cells frozen 1 day, 74 ± 2, 17 ± 2, 9 ± 2; cells frozen 10 days, 73 ± 2, 18 ± 1, 9 ± 1; and cells frozen 3 months, 70 ± 2, 18 ± 1, 12 ± 2.

FMF Analysis of DNA Distributions

DNA distributions were assessed with a multiparameter cell sorter/analyzer built according to the design of Steinkamp et al (14), but modified as described by Pinkel et al (15). The fluorescence intensity (DNA content) of $\geq 10^4$ cells was determined, and cell numbers in each of 256 fluorescence intensity channels were recorded using a PDP 11/10 minicomputer (Digital Equipment Corp, Maynard, MA). DNA distributions were analyzed with the minicomputer utilizing the functional form proposed by Dean and Jett (7). The background was represented by a hyperbola. The G_1-G_0 and G_2 + M peaks were represented by Gaussian distributions, and the S-phase region was represented by a sum of approximately 30 Gaussians (the exact number used depended on each individual distribution) weighted by a second-order polynomial. The S-phase region was chosen so that the mean of the first Gaussian used in representing it was 1 SD beyond the mean of the

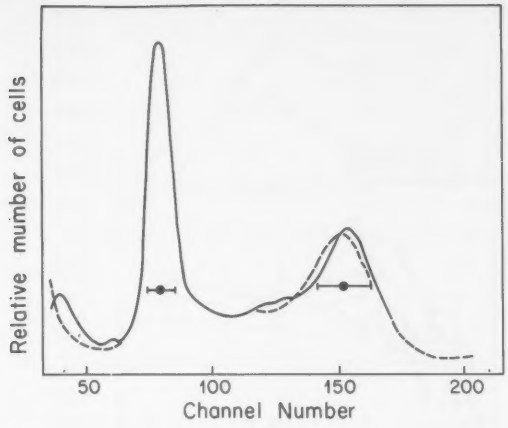

FIGURE 1.—DNA distribution of LA-N-1 neuroblastoma cells grown in vitro. DNA content of cells is proportional to fluorescence intensity. Both the actual data curve (———) and the computer fit curve (----) are graphed. The peak around channel 76 consists primarily of G_1-G_0 cells, and the peak around channel 152 consists primarily of G_2 + M cells. The region between these peaks is populated by cells in S phase. The means ± 1 SD of the G_1-G_0 and G_2 + M peaks are indicated (—●—).

G_1-G_0 peak and the mean of the last Gaussian was 1 SD before the mean of the G_2 + M peak (fig 1). A nonlinear least-squares fitting algorithm was used to compute the optimum parameters of all these terms. The areas under the G_1-G_0 and G_2 + M Gaussians as well as under the S-phase region were computed and used to determine the percentage of cells in each phase.

RESULTS

Cytotoxic Effects of Cyclophosphamide on LA-N-1 and SK-N-MC Cells

Cyclophosphamide was more cytotoxic for SK-N-MC cells than for LA-N-1 cells both in vitro and in vivo (fig 2). Pulse treatment of LA-N-1 cells in vitro with liver S-9-activated cyclophosphamide (10 μg/ml) caused a 54% decrease in cell numbers 48 hrs later and, in separate tests, caused a 57% ± 22% (mean ± 1 SD, nine experiments) decrease in colony-forming cells. The size of LA-N-1 tumors in nude mice was not significantly decreased by cyclophosphamide (250 mg/kg ip). By contrast, S-9-activated cyclophosphamide (10 μg/ml) caused an 85% decrease in SK-N-MC cells 48 hrs later and, in other tests, caused a > 95% decrease in colony-forming cells (ten experiments). In vivo, cyclophosphamide (250 mg/kg ip) reduced tumor size by > 50% in nearly all mice, but all tumors regrew.

DNA Distributions of LA-N-1 Cells After Treatment With Cyclophosphamide

A representative DNA distribution of untreated LA-N-1 cells and results of computer analysis of the distribution are shown in figure 1. The proportion of cultured cells in G_2 + M was markedly increased 24 and 48 hrs after pulse treatment with liver S-9-activated cyclophosphamide (10 μg/ml), and even greater increases in G_2 + M cells occurred following treatment with 100 μg/ml of cyclophosphamide (fig 3). Concomitant with these increases in G_2 + M cells, the proportion of G_1 cells decreased. Cells analyzed in these in vitro experiments were adherent and able to exclude trypan blue, and at least some retained proliferative capacity since their number was increasing by 96 hrs and they were able to form colonies.

DNA distributions of LA-N-1 cells in nude mice after cyclophosphamide were similar although not identical to those of cultured cells after cyclophosphamide (fig 4). Two days after a single dose (250 mg/kg), the percent of G_2 + M and S cells increased and the percent of G_1 cells decreased. At 4 and 6 days, proportions of G_2 + M cells increased further, and G_1 cells concomitantly decreased. S-phase cells again increased at 8 days. All cycle compartments returned to control levels by 10 days after therapy. These changes occurred even though the sizes of LA-N-1 tumors were stable or increasing (fig 2).

DNA Distributions of SK-N-MC Cells After Treatment With Cyclophosphamide

SK-N-MC cells surviving treatment with liver S-9-activated cyclophosphamide (10 μg/ml) were adherent and able to exclude trypan blue; some retained proliferative potential since their numbers increased by 72 hrs and they were able to form a few colonies. The G_2 + M population was decreased fivefold at 24 and 48 hrs; however, by 72 hrs, it was not significantly different from untreated cells (fig 5). Higher doses of cyclophosphamide in vitro were so cytotoxic that adequate numbers of cells could not be obtained for analysis. In vivo, one injection of cyclophosphamide caused partial regression (> 50% decrease in size) of SK-N-MC tumors in nearly all mice (fig 2). At 2–6 days, there was a fivefold decrease in the G_2 + M cells and an increase in G_1 cells (fig 6). These populations returned to pretreatment levels by 10 days after therapy when tumors were beginning to regrow.

DISCUSSION

Although cytotoxic effects of cyclophosphamide on neuroblastoma cells are appreciated from clinical

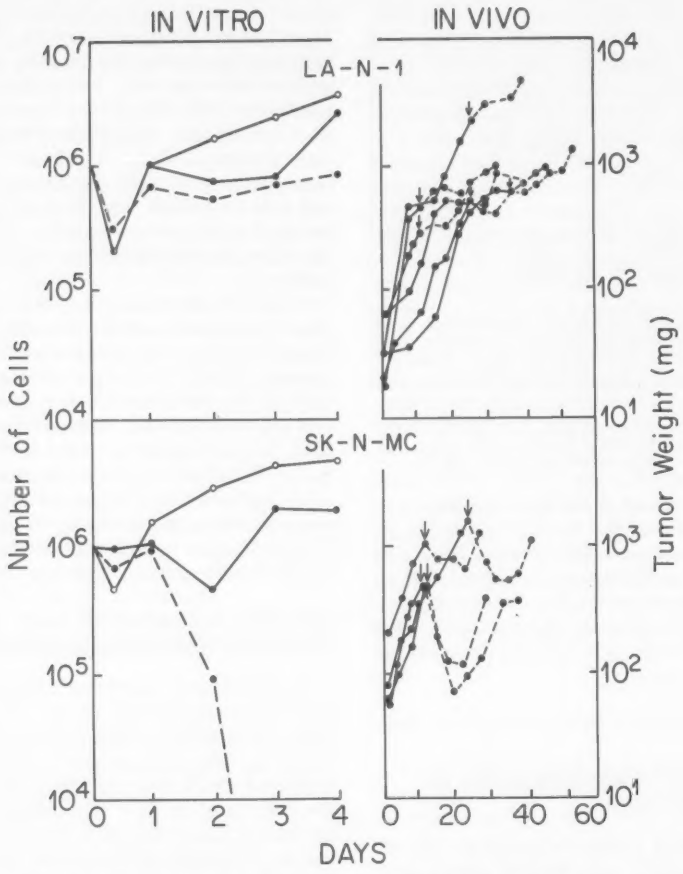

FIGURE 2.—Effect of cyclophosphamide on LA-N-1 and SK-N-MC neuroblastoma cells growing in vitro and in nude mice. In vitro, 10^6 cells were pulse-treated with liver S-9-alone (○——○) or with liver S-9-activated cyclophosphamide (10 μg/ml, ●——●; 100 μg/ml, ●----●), plated, and counted at indicated times. Cells enumerated were adherent and able to exclude trypan blue. No SK-N-MC cells treated with 100 μg/ml survived after 2 days. In vivo, each animal was given a single dose of cyclophosphamide (250 mg/kg ip) on the day indicated by the arrow. Tumor weight (mg) = $\frac{\text{length} \times \text{width}^2}{2}$. Data are from representative experiments.

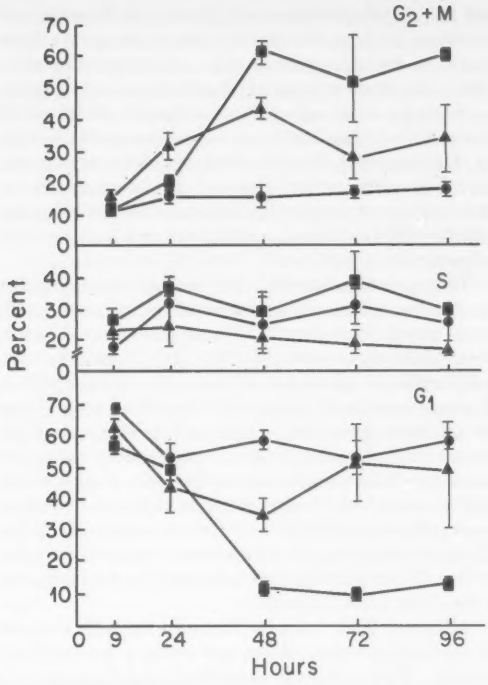

FIGURE 3.—Effect of cyclophosphamide on DNA distribution of LA-N-1 cells in vitro. % (mean ± 1 SD) of cells in G_2 + M, S, and G_1 at 9–96 hrs after pulse treatment with liver S-9 alone (●——●) or liver S-9-activated cyclophosphamide (10 μg/ml, ▲——▲; 100 μg/ml, ■——■) is indicated. Cells analyzed were adherent and able to exclude trypan blue. Each data point is the result of 3–5 experiments.

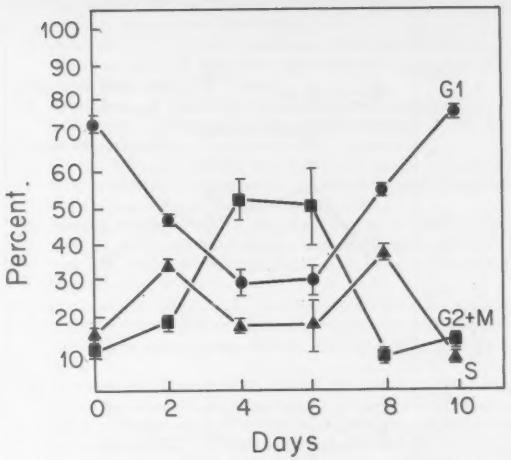

FIGURE 4.—Effect of cyclophosphamide on DNA distribution of LA-N-1 cells growing as subcutaneous tumors in athymic nude mice. When tumors reached 10 mm in diameter, mice were given 1 injection of cyclophosphamide (250 mg/kg ip). 2–10 days afterwards, mice were killed, tumors were removed and dissociated, and cells were analyzed. % (mean ± 1 SD) of cells in G_1 (●——●), S (▲——▲), and G_2 + M (■——■) is indicated; each data point is the result of analysis of 3–5 tumors. There was no effect of tumor size (6–22-mm diameter) on DNA distributions for untreated tumors (data not shown).

studies (2,5), few data are available to indicate effects of this drug on neuroblastoma cell cycle distributions (5). Using human neuroblastoma cell lines LA-N-1 and SK-N-MC, which are relatively resistant and sensitive, respectively, to cyclophosphamide, we demonstrated that a single dose of cyclophosphamide can induce marked perturbations in DNA distributions that are significantly different for the two cell lines.

The proportion of LA-N-1 cells in G_2 + M increased and the proportion of those in G_1 decreased after one dose of cyclophosphamide, even though cytotoxic effects were moderate in vitro and negligible in vivo. The increase in G_2 + M cells appears to be primarily due to G_2 cells since no increase in M cells was noted from careful histologic examination of treated tumors (data not shown); an increased proportion of G_2 cells could be due to a delay in progression during G_2 or to a selective cytotoxic effect against G_1 cells. In vivo, the percent of S-phase cells was increased 2 and 8 days after cyclophosphamide. This may reflect recruitment from G_0 or G_1 phases at 2 days and initial proliferative recovery at 8 days. Detection of changes in DNA distributions of LA-N-1 cells in the absence of reduction in tumor size is important because it suggests that DNA distribution analysis may be a sensitive means of monitoring response to therapy.

The cytotoxic and cytokinetic effects of cyclophosphamide on SK-N-MC cells were strikingly different from those on LA-N-1 cells. Most SK-N-MC cells were killed by cyclophosphamide, and the survivors were predominantly in G_1 and S phases with few in G_2 + M. Such alterations in DNA distributions have not previously been observed after cyclophosphamide. Some of the surviving cells may have been delayed or arrested in G_1; alternatively, G_2 + M cells may have been relatively more susceptible to lethal effects of cyclophosphamide. Finally, surviving "G_1" cells actually could be relatively resistant G_0 cells capable of re-entering the cell cycle.

The cells analyzed with FMF undoubtedly were heterogeneous with regard to proliferative poten-

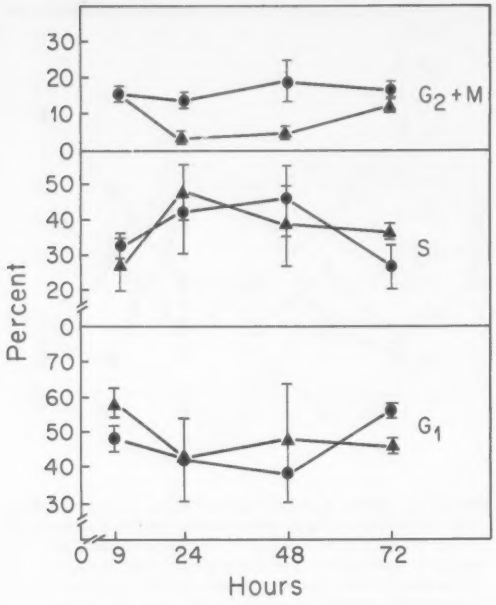

FIGURE 5.—Effect of cyclophosphamide on DNA distribution of SK-N-MC cells in vitro. % (mean ± 1 SD) of cells in G_2 + M, S, and G_1 9–72 hrs after pulse treatment with liver S-9 alone (●——●) or liver S-9-activated cyclophosphamide (10 μg/ml, ▲——▲) is shown. Cells analyzed were adherent and able to exclude trypan blue. Each data point is the result of 3–5 experiments.

tial after cyclophosphamide, particularly when cell numbers and tumor size were decreasing. At these times, some were terminal even though they were able to exclude trypan blue and adhere to surfaces, and others were capable of continued proliferation since cell number and tumor size soon increased (fig 2). Determining the distribution of clonogenic and terminal cells within the cell cycle after chemotherapy is of major importance for combination therapy and may be possible with cell-sorting techniques.

Two previous studies of rodent tumors growing in vivo determined the effects of cyclophosphamide on cell cycle distribution. Using the labeling index, Braunschweiger and Schiffer (16) found that the proportion of spontaneous mammary tumor cells in S phase increased within 24 hrs after a single dose of cyclophosphamide; then cell proliferation decreased for a variable period and finally increased prior to initiation of tumor regrowth. Using FMF, Alberts and Van Daalen Wetters (17) demonstrated accumulation of P388 murine leukemia cells in the G_2 phase following a single dose of cyclophosphamide. These effects are comparable to those observed for LA-N-1 cells.

Although FMF may facilitate cytokinetic analysis of neuroblastomas, there are some potential limitations. These include identification of tumor cells when they are mixed with normal cells such as in bone marrow, cell kinetic heterogeneity within the primary tumor and between the primary and its

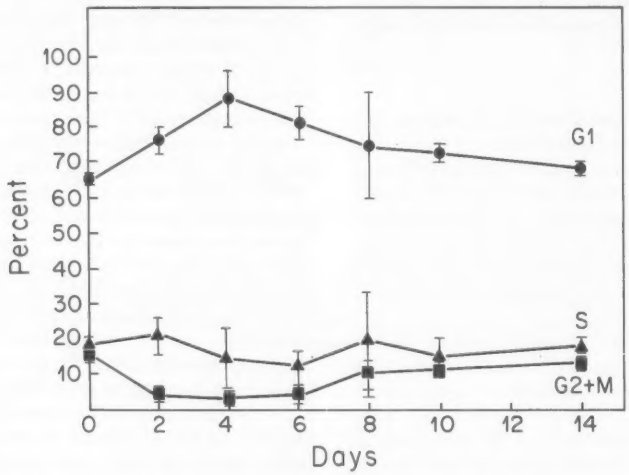

FIGURE 6.—Effect of cyclophosphamide on DNA distribution of SK-N-MC cells growing as subcutaneous tumors. % (mean ± 1 SD) of cells in G_1 (●——●), S (▲——▲), and G_2 + M (■——■) 2–14 days after cyclophosphamide (250 mg/kg ip) is indicated. Each data point is the result of analysis of 3–5 tumors. There was no effect of tumor size (6–22-mm diameter) on DNA distributions for untreated tumors (data not shown).

metastases, and identification of G_0 cells. We currently are developing a method to identify tumor cells when they are mixed with normal cells that utilizes fluoresceinated antitumor antibodies (18). The extent of kinetic heterogeneity in a tumor and its metastases can be determined only by analyzing samples of tumors. Assessment of the proportion of noncycling cells currently depends upon the primer-dependent DNA polymerase method (16) and upon new staining procedures for FMF (19,20).

In conclusion, we have found that cyclophosphamide induces marked but different alterations in the DNA distributions of two human neuroblastoma cell lines. Recent evidence of Hayes et al (5) indicates that cyclophosphamide also perturbs cell kinetics in some, but not all, neuroblastomas in children. Their study and our studies suggest that heterogeneity in the cytokinetic response of neuroblastoma cells to cyclophosphamide may be a general phenomenon. Additional experimental and clinical studies are necessary to determine the extent of such heterogeneity. Definition of cell kinetic responses may be of prognostic importance and may also lead to appropriate and more successful combination chemotherapy.

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Lactic Acidosis Associated With Metastatic Breast Carcinoma[1]

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SUMMARY

Occurrence of lactic acidosis with adequate tissue oxygenation (type B lactic acidosis) has been described in association with leukemia, lymphoma, and a single case of Hodgkin's disease. No cases of this type have been reported in association with solid tumors. A case of type B lactic acidosis in a woman with rapidly progressing metastatic breast carcinoma is described.

[Cancer Treat Rep 64:1283–1285, 1980]

Lactic acidosis occurs in two types of clinical settings. In type A lactic acidosis, inadequate tissue oxygenation is clinically evident as in shock or severe hypoxemia. In type B lactic acidosis, adequate tissue oxygenation appears to be present and lactic acidosis is the result of a defect in metabolism induced by an inherited metabolic disorder, by ingestion of a drug such as phenformin, or by some unidentified interference with lactate metabolism (1,2). Type B lactic acidosis has been described in hematologic malignancies, particularly leukemia and lymphoma (3,4) and in a single case of Hodgkin's disease (5). So far, to our knowledge, there are no reported cases of lactic acidosis in association with solid tumors. We describe here the occurrence of lactic acidosis in association with metastatic adenocarcinoma of the breast.

CASE REPORT

A 61-year-old white female was admitted to the University of Wisconsin Hospitals in May 1978 with progressive weakness, dyspnea, and a nonproductive cough. In September 1976, she underwent a modified radical mastectomy for adenocarcinoma of her left breast. The axillary lymph nodes were uninvolved. She received no further treatment. In early 1977, a clinical diagnosis of sarcoidosis was made because of bilateral basal interstitial infiltrates and diminished diffusion capacity with mild hypoxia. She improved with prednisone treatment, and, at the time of hospitalization in May 1978, she was given 10 mg of prednisone every other day. Because of development of nodular densities in upper lung fields in addition to basal interstitial infiltrates, a transbronchial lung biopsy was performed in January 1978 and showed multiple noncaseous epithelioid granulomas, substantiating the diagnosis of sarcoidosis. She was discharged and continued to take prednisone at a dose of 10 mg every other day, and was re-admitted in May 1978. The patient had no previous hepatic, renal, or cardiac problems.

At admission, the patient looked ill; her blood pressure was 110/60 mm Hg without postural change. Abdominal examination revealed an enlarged, firm, nodular liver with total span of 16 cm. No ascites or peripheral edema was present. The remainder of her physical examination was unremarkable. Laboratory data on May 31, 1978, included hematocrit of 36%, wbc count of 14,500/mm^3, BUN of 25 mg/100 ml, uric acid of 9.7 mg/100 ml, bilirubin of 1.8 mg/100 ml, serum LDH of 295 IU (normal, 100–210 IU), serum alkaline phosphatase of 316 IU (normal, 20–110 IU), SGOT of 81 units (normal, up to 35 IU), and serum albumin of 3.2 g/100 ml with total proteins of 5.2 g/100 ml. Electrolyte values

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[4]We thank Frances Kluter for her excellent secretarial assistance.

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were: sodium, 141 mEq/liter; potassium, 5.1 mEq/liter; CO_2 content, 19 mEq/liter; and chloride, 103 mEq/liter (anion gap, 19 mEq/liter). Arterial blood gases on room air revealed P_{O_2}, 81 mm Hg; P_{CO_2}, 27 mm Hg; pH 7.39; and actual bicarbonate, 15.5 mEq/liter. Urinalysis revealed a specific gravity of 1.002 and pH 6; protein, ketones, and blood were negative. Microscopic examination was unremarkable. A chest roentgenogram showed multiple nodular densities in upper lung fields and bilateral basal interstitial infiltrates which were unchanged from the previous examination in February 1978. Liver and spleen radionucleotide scan showed markedly enlarged liver with multiple filling defects. A percutaneous liver biopsy showed metastatic adenocarcinoma.

Initially, the patient was treated with 60 mg of prednisone for sarcoidosis and dietary salt restriction. After 1 week she was noted to have significant leg and sacral edema and a hard, fixed nodule over the right antecubital fossa. Significant orthostatic tachycardia without change in blood pressure was present. Laboratory data 8 days after admission (June 8, 1978) showed the following: BUN, 56 mg/100 ml; creatinine, 1.2 mg/100 ml; uric acid, 12 mg/100 ml; and bilirubin, 2.1 mg/100 ml. Electrolyte values were: sodium, 136 mEq/liter; potassium, 6.3 mEq/liter; CO_2 content, 18 mEq/liter; and chloride, 98 mEq/liter. Urine sodium was 8 mEq/liter, urine potassium was 56 mEq/liter, and urine osmolality was 592 mOsm/liter. The patient was treated for volume depletion by iv saline infusions; orthostatic tachycardia then disappeared and the BUN fell towards normal. Her hyperkalemia was successfully treated with Kayexalate enemas for 2 days. She continued to have edema of the legs and sacral region. Her acidosis worsened in spite of oral sodium bicarbonate given at a dose of 1 g four times per day. Serum acetone remained negative. Plasma lactate levels on two occasions 3 days after correction of hypovolemia were 15 mEq/liter and 17.2 mEq/liter (normal, 0–2 mEq/liter). On the 13th hospital day arterial blood gases revealed the following: P_{O_2}, 91mm Hg; P_{CO_2}, 21mm Hg; pH, 7.31; and actual bicarbonate, 10.1 mEq/liter. Electrolyte values were: sodium, 136 mEq/liter; potassium, 4.6 mEq/liter; CO_2 content, 10 mEq/liter; chloride, 97 mEq/liter; BUN, 38 mg/100 ml; and creatinine, 1.1 mg/100 ml. The patient started receiving chemotherapy at the end of the first week of hospitalization, after the diagnosis of metastatic liver disease was made by liver biopsy. The skin nodule that appeared 1 week after admission was presumed to be metastatic; however, it was not biopsied. On the 13th day of hospitalization the patient signed out of the hospital, against medical advice.

DISCUSSION

Lactic acid is an important byproduct of glycolysis. Pyruvate and lactate are also important precursors in the formation of glucose by the liver and the kidney. The major tissues producing lactate are skin, erythrocytes, and skeletal muscle. Although lactate is metabolized in almost all the tissues, liver and kidney are the main tissues involved in lactate clearance and utilization. The liver can clear up to several thousand millimols of lactate per day (6,7) while normal turnover is about 1500 mmols/day (8). Lactic acidosis develops when the rate of production of lactate exceeds the rate of elimination. In type B lactic acidosis, which occurs in the presence of adequate tissue oxygenation, lactate overproduction is one of the more common causes of lactic acidosis. In addition, hepatic derangement would contribute to the lactic acidosis by decreasing the lactate clearance.

Our patient fits clearly into this category of type B lactic acidosis. The patient presented with metabolic acidosis, increased anion gap, and markedly elevated blood lactate levels. Other causes of lactic acidosis were eliminated from the history and laboratory data. Initial hypovolemia is probably not a contributing factor in the patient's lactic acidosis because her acidosis worsened gradually, in spite of correction of hypovolemia. Apart from the diagnosis of metastatic cancer and sarcoidosis, the patient had no past renal hepatic or cardiac problems. There was neither a history of a chemical or drug ingestion nor did the patient receive hypertonic glucose solutions during the hospitalization (9) that could account for lactic acidosis. We have not found any reported association between sarcoidosis and lactic acidosis. Also, since her sarcoidosis was not worse, either clinically or radiologically compared to the previous year, it is highly unlikely that sarcoidosis was responsible for the lactic acidosis. There have been reports of the occurrence of lactic acidosis in leukemia and lymphoma. It is probable that, in this case, metastatic carcinoma was the cause of the lactic acidosis, sarcoidosis being a coincidental problem.

The cause of lactic acidosis in leukemia and lymphoma is not clearly understood. Field et al (3) postulated that leukemic cells in massive number get overcrowded in various organs so that they become poorly oxygenated and produce lactic acid by their dependence on anaerobic glycolysis. They showed that leukemic cells in vitro produce lactic acid in large quantities only in an anaerobic environment. A similar situation may also pertain to the case of solid tumors with rapid proliferation. The rapid growth of the tumor cells may outstrip their blood

supply leading to anaerobic metabolism in tumor tissue. In addition, the patient's metastatic carcinoma involved large areas of liver and this may have decreased the lactate clearance by the liver. However, if the tumor replacement of liver was so massive as to impair lactate metabolism, more derangement in hepatic handling of bilirubin than was exhibited by this patient might have been expected.

It is likely that the high urate level in this patient reflected high nucleic acid turnover in the tumor itself. However, the occurrence of hyperuricemia with the onset of lactic acidosis is interesting since it was shown that high lactate levels inhibit tubular secretion of uric acid (10).

In summary, a case of type B lactic acidosis was observed in a woman with metastatic breast carcinoma with massive hepatic involvement and coincidental sarcoidosis. This appears to be the first report of the occurrence of type B lactic acidosis in association with a solid tumor.

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Dianhydrogalactitol and Neural Tumors: An In Vitro, In Vivo Preclinical Evaluation[1,2]

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SUMMARY

The effects of dianhydrogalactitol on human neuroblastomas in vitro and in vivo as heterotransplants in nude mice were determined. Four neuroblastoma lines and three primitive neuroectodermal tumor lines were found, in vitro, to have different sensitivities to the drug. The most sensitive in in vitro assays was the neuroblastoma line SK-N-Mc. Tumors from patients resistant to cyclophosphamide were sensitive to dianhydrogalactitol in vitro and in nude mice. The lack of increased cytotoxicity in vitro with concentrations > 12 μg/ml and the lowered degree of weight loss in mice treated with 6 mg/kg/day × 5 consecutive days compared to 15 mg/kg/day × 2 days (either in sequence or with a 3-day interval) suggest that clinical trials with 5-day courses or constant infusions may be more effective than intermittent pulsed doses.

[Cancer Treat Rep 64:1287–1294, 1980]

Halogenated hexitols are converted in serum to diepoxides which have antitumor activity (1). One of these hexitols, dibromodulcitol, is converted to its diepoxide 1,2:5,6-dianhydrogalactitol (DAG). This compound was found to have antitumor effects on HeLa cells (2) and animal tumors, including B16 melanoma, ependymoma, and rhabdomyosarcoma.[4] Dose schedules consisting of a 5-day course given at least twice or single weekly doses have been used, with a limited number of responses observed in patients with renal cell carcinoma, adenocarcinoma of the lung, and laryngeal carcinoma (3–6). The observation of antitumor effect on ependymomas in animals suggests that DAG might have activity against other neural tumors. Accordingly, we tested its effects on human neuroblastomas and primitive neuroectodermal tumors both in vitro and in vivo as heterotransplants in nude mice. These tumors were chosen because they represent common neural tumors of childhood.

MATERIALS AND METHODS

Cell Cultures

Neuroblastoma cell lines [SK-N-Sh (7), SK-N-Lo,[5] and SK-N-Dz[5]] and cell lines derived from primitive malignant neuroectodermal tumors [SK-PN-Ke,[5] SK-PN-SC,[5] and SK-PN-Wa[5]] were tested. The cell lines SK-N-Sh, SK-N-Lo, and SK-N-Dz were derived from metastatic neuroblastoma tumors present in the bone marrow of three different patients. SK-N-Mc was derived from a metastatic neuroblastoma tumor of the orbit. The primitive neuroectodermal tumors SK-PN-Ke and SK-PN-SC were established from biopsy material of tumors located in the brain, and SK-PN-Wa was derived from a biopsy of a tumor of the posterior thorax.

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[3]Pediatric Cancer Research Laboratory, Memorial Sloan-Kettering Cancer Center, New York, NY.

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[5]These tumor lines have been established; manuscripts are in preparation.

TABLE 1.—Effects of DAG on neural tumor cells in vitro

Cell line*	No. of cells inoculated	No. of cells (±SE) at 96 hrs†		P value‡	% cell kill	% growth inhibition§
		Untreated	Treated			
SK-N-Sh	1000	9307 ± 1209	3611 ± 421	0.001		75.3
SK-N-Dz	2000	7825 ± 514	2232 ± 139	0.001		96.0
SK-N-Lo	3000	7368 ± 218	2806 ± 162	0.001	7.0	
SK-N-Mc	2000	7228 ± 120	225 ± 160	0.001	90.0	
SK-PN-Wa	2000	6464 ± 105	4635 ± 460	0.01		41.0
SK-PN-Sc	2000	3778 ± 456	2950 ± 232	NS¶		46.0
SK-PN-Ke	2000	3493 ± 30	2467 ± 78	0.001		68.7

*Cell lines: SK-N = neuroblastoma; SK-PN = primitive neuroectodermal tumors.

†Cells were exposed to 20 μg/ml of DAG.

‡Based on comparison of the means of the No. of human neuroblastoma cells in 4 control wells and 4 treated wells using Student's t test.

§% kill was determined when the No. of cells at termination of the study was less than the No. of cells inoculated into the wells. Cell loss is assumed to result from 100% growth inhibition + actual cell death. % inhibition refers to the increment of treated cells, compared with the increment of untreated controls.

¶NS = no significant difference.

After removal from the patient, all specimens were immediately placed in medium and processed according to methods previously described (7). The pathologic designation, primitive neuroectodermal tumor, is now being applied to tumors which are similar to embryonal rhabdomyosarcoma and Ewing's sarcoma but which have different clinical and morphologic characteristics.

In Vitro Tumor Cell Assay

Cells were grown in Eagle's MEM plus 15% fetal calf serum and incubated in an atmosphere of 5% CO_2 in air at 37°C. At every fourth passage, all cell lines were tested for and remained free of mycoplasma contamination. Growth inhibition assays were conducted in Falcon 96-well micrometer plates (Falcon Plastics, Oxnard, CA). Four wells for each dose or time of drug exposure and four wells for controls were used throughout these experiments, and each assay was run twice; 1000–3000 cells in 0.1-ml volumes were plated in replicate wells. Twenty-four hours after plating, cell monolayers formed, and the cells began their log phase of growth. At this time, DAG was dissolved in media immediately prior to use, and 0.1-ml volumes of appropriate concentrations were added to each of the test wells. Drug exposures ranged from 15 minutes to 96 hours. Short exposures were made by aspirating DAG-containing medium from each well after appropriate intervals and replacing it with fresh medium. Tumor growth inhibition was determined by harvesting the cells, using an electronic particle counter (Coulter Electronics, Hialeah, FL). The termination time for in vitro assays was either 96 hours for continuous drug exposures (table 1 and fig 1) or 168 hours for varying exposure periods (table 2). Before harvesting the cells, the floating

TABLE 2.—Effects of exposure duration to DAG in vitro*

Dose (μg/ml)	Exposure duration (hrs)	% cell kill	% growth inhibition
1	0.25		13
5	0.25		16
10	0.25		29
20	0.25		49
20	1.0		82
20	2.0		88
20	4.0		88
20	6.0		92
20	96		96.6
20 × 2†	0.25 × 2	51.0	

*Values = the mean No. of human neuroblastoma SK-N-Mc cells of 4 wells compared with untreated controls after 7 days' growth.

†In 1 experiment, cells were exposed to DAG for 15 mins twice at 2-day intervals.

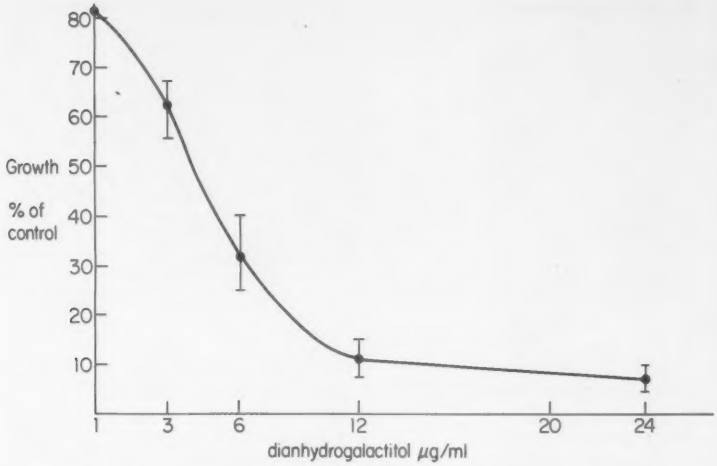

FIGURE 1.—Effects of DAG on growth of human neuroblastoma cells SK-N-Mc exposed for 96 hrs in vitro. DAG concentrations between 1 and 12 μg/ml cause a steep sloped growth inhibitory curve, while concentrations > 12 μg/ml have little additional effect.

cells in control and test wells were tested for viability by placement in fresh media and counting attached cells after an additional 7-day incubation. Since the number of floating viable cells never exceeded 5% of the total number of cells, this factor was not included in the calculations of cell growth inhibition. The following calculations for percent tumor cell growth inhibition and cell kill were used:

A = number of cells plated;
B_c = number of untreated cells after 96 hours;
B_t = number of treated cells after 96 hours;

$$B_c = \frac{B_c - A}{A} \times 100 = \text{percent change for untreated cells;}$$

$$B_t = \frac{B_t - A}{A} \times 100 = \text{percent change for treated cells; and}$$

$$100 - \frac{B_t}{B_c} \times 100 = \text{percent growth inhibition}$$

$$100 - \frac{B_t}{A} \times 100 = \text{percent tumor cell kill where } B_t = A.$$

Heterotransplant Tumor Assay

Nude mice were obtained from the Animal Facilities of the Memorial Sloan-Kettering Cancer Center. They were of an outbred Swiss background. Females 6–8 weeks old were inoculated sc in the right-shoulder region with 5×10^6 SK-N-Mc and 3×10^6 SK-N-Dz cultured tumor cells. Sample size varied from seven or eight mice each for SK-N-Mc control and treatment groups to four each for SK-N-Dz control and treatment groups. The latter tumors grew with less variability in control mice; hence, smaller groups could be used. When tumor diameters reached 0.5–1.0 cm, treatment with DAG was started. The drug was weighed and diluted in saline to appropriate concentrations immediately prior to ip administration. Drug doses were 15 mg/kg/day × 2 days in sequence or with a 3-day interval or 4–6 mg/kg/day × 5 days.

On all mice, measurements of the two greatest tumor dimensions were taken with calipers daily for the first week and every third day thereafter. After the tumor area was computed for a given day, the relative tumor area (RTA) was computed by dividing the area for that day by the area computed at the start of the trial. The RTA was plotted against days and, for each mouse, the area under the RTA curve was computed. "Large" areas indicate little treatment effect; "small" areas indicate inhibition of tumor growth by treatment (8). The variability in measurements and growth rates of control and treated animals are accounted for in the statistical method where the coefficient of variation is incorporated in determining the number of animals needed in each group and in calculating the significance of the differences between treated and control groups.

RESULTS

In vitro studies with DAG revealed the neuroblastomas to be the most sensitive of the cell lines

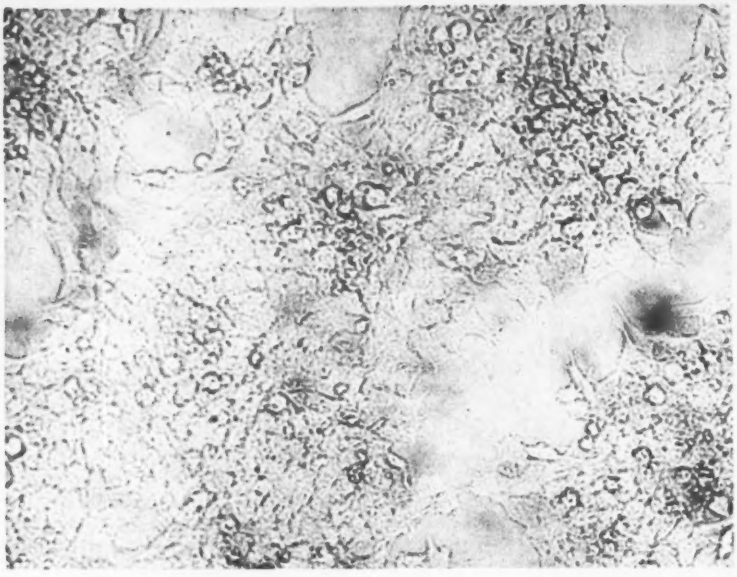

FIGURE 2.—Neuroblastoma cells SK-N-Mc, untreated controls, growing as a monolayer. Lightfield × 130.

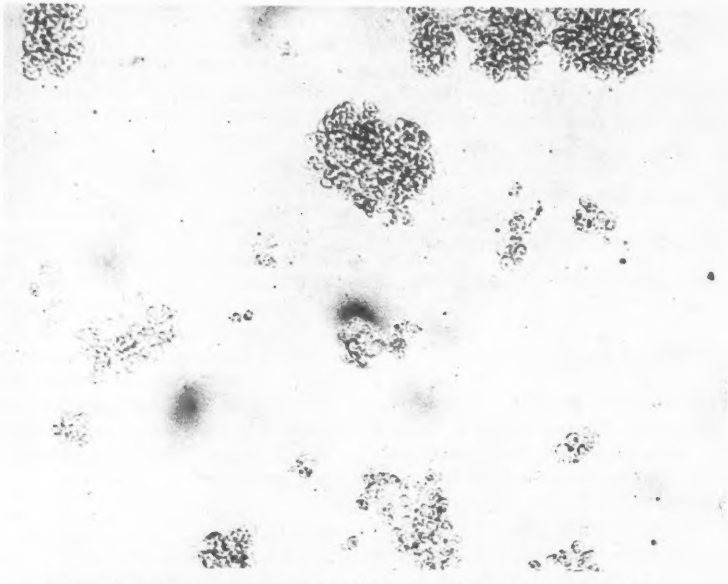

FIGURE 3.—Neuroblastoma cells SK-N-Mc exposed for 24 hrs to 20 μg/ml of DAG. Virtually all cells were necrotic. Lightfield × 130.

TABLE 3.—Statistical analyses of effects of DAG on human neuroblastoma SK-N-Mc heterotransplanted to nude mice*

Treatment	Mean RTA (mean $\sqrt{RTA}$)†	SD	Coefficient of variation	Sample size (No. of mice)
Control	17.67 (12.58)	11.03 (3.40)	0.62 (0.27)	7
5-day DAG	4.44 (6.27)	0.62 (0.5)	0.14 (0.8)	8

*According to the Wilcoxon rank-sum test, there was no difference in the initial absolute size of tumors between the control and treated groups.

†There is a significant difference between the area under the curve RTA or the $\sqrt{RTA}$ of treated and untreated groups, using the Kruskal-Wallis nonparametric ANOVA ($P = 0.0001$), ref 8.

tested. In these experiments, if the cell counts at the termination of the experiment were less than the number of cells actually plated, the effect was considered to represent cell kill. If the increment in the number of treated cells was less than the increment in untreated controls but greater than the number of plated cells, the effect was considered to be growth inhibition (table 1). Analysis after a 7-day growth period showed that exposures to 20 μg/ml of DAG for as short a time as 15 minutes were sufficient to cause significant growth inhibition (table 2). With continuous 72-hour exposures, the cells were responsive in an almost linear fashion to concentrations of DAG between 1 and 12 μg/ml; however, significant additional cell growth inhibition was not observed with concentrations > 12 μg/ml (fig 1). Except for an increased number of pyknotic and amorphous cells, typical changes of neuroblastoma cell morphology such as process formation were not observed in any treated cells (figs 2 and 3). DAG-induced tumor regressions in vivo were observed with the heterotransplanted tumors SK-N-Mc and SK-N-Dz (table 3 and figs 4 and 5).

Within 48 hours after the first dose of DAG, SK-N-Mc tumors began to regress. Weight loss accompanied tumor regressions in mice treated with the schedule of 15 mg/kg/day × 2 in sequence or with a 3-day rest period but not in the animals treated with 6 mg/kg/day × 5. The weight loss ranged from 5% to 25%, and it developed within 48–72 hours after the first injection of DAG. There were four deaths among 20 mice with 20%–25% weight loss, who were treated on one or the other of the 2-day schedules. At autopsy, congested livers and spleens smaller than usual were noted. No evidence of gastrointestinal tract hemorrhage was detected. These treated tumors continued to regress after treatment was stopped, and total elimination of SK-N-Mc and SK-N-Dz tumors was observed by the third week following treatment. Animals formerly bearing SK-N-Mc tumors continue to remain free of tumor, without recurrence 16 weeks after therapy was given. Tumors in control animals became excessive in size, and all tumor-bearing untreated controls died or eventually were killed because of excess tumor size (table 3). Animals with SK-N-Dz tumors also showed good tumor regressions at 15 mg/kg/day × 2 and 6 mg/kg/day × 5; however, tumor recurrences were observed at the lower doses 35–40 days after treatment was started (figs 4 and 5).

DISCUSSION

These data extend to human neural tumors the original observations of cytotoxic effects of DAG on murine ependymoma and melanoma tumors (3). In vitro studies of exposures to DAG of 0.25–96 hours revealed that neuroblastoma cells were generally more sensitive than primitive neural cell tumors. On the basis of its chemical activity and cross-resistance to alkylating agents, the mechanism of action of DAG was reported to be an alkylation (9, 10). In these studies, we found paradoxical observations concerning cross-resistance between DAG, cyclophosphamide, neuroblastoma, and primitive neuroectodermal tumors. The four neuroblastoma cell lines were derived from patients who were treated with and exhibited resistance to cyclophosphamide at different dose levels. All these patients exhibited brief responses to cyclophosphamide, but with continued administration, these tumors eventually regrew. In the patients from whom SK-N-Mc and SK-N-Sh were derived, the doses were not escalated beyond 20 and 40 mg/kg at the time of relapse. In the patients from whom SK-N-Dz and SK-N-Lo were derived, doses were escalated to 160 mg/kg given over 2 days, without tumor response. At that time in the course, bone marrow aspirates were used to establish these cell lines (table 4). The cyclophosphamide exposure of the patient from whom the SK-N-Mc tumor line was derived was much less than that of the patient from whom the

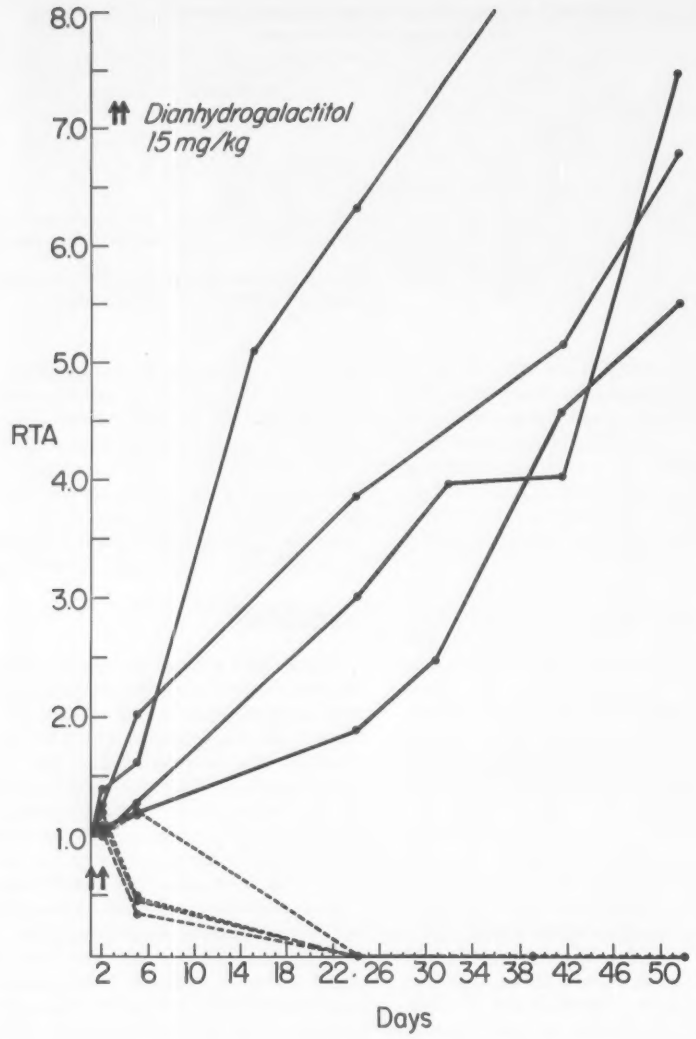

FIGURE 4.—Effect of DAG injected ip at 15 mg/kg/day on 2 successive days into nude mice bearing human neuroblastoma SK-N-Dz. Solid lines = controls. Dashed lines = treated mice. Each line = 1 mouse. Complete tumor lysis in all 4 treated mice was seen with this schedule; however, it was accompanied by important weight loss and death in 2 mice.

SK-N-Dz was derived. The former appeared to be more sensitive to DAG than the latter, although both tumors were sensitive to DAG in vitro and in nude mice. It should be emphasized that there is a good correlation between sensitivity to cyclophosphamide of neuroblastomas in the clinical setting and as heterotransplants in nude mice (11, 12). The primitive neuroectodermal tumors, on the other hand, were observed to be relatively insensitive to DAG under in vitro conditions. None of the three patients from whom these tumors were derived exhibited any response to cyclophosphamide. This suggestion of cross-resistance is emphasized in the patients from whom SK-PN-Sc and SK-PN-Wa tu-

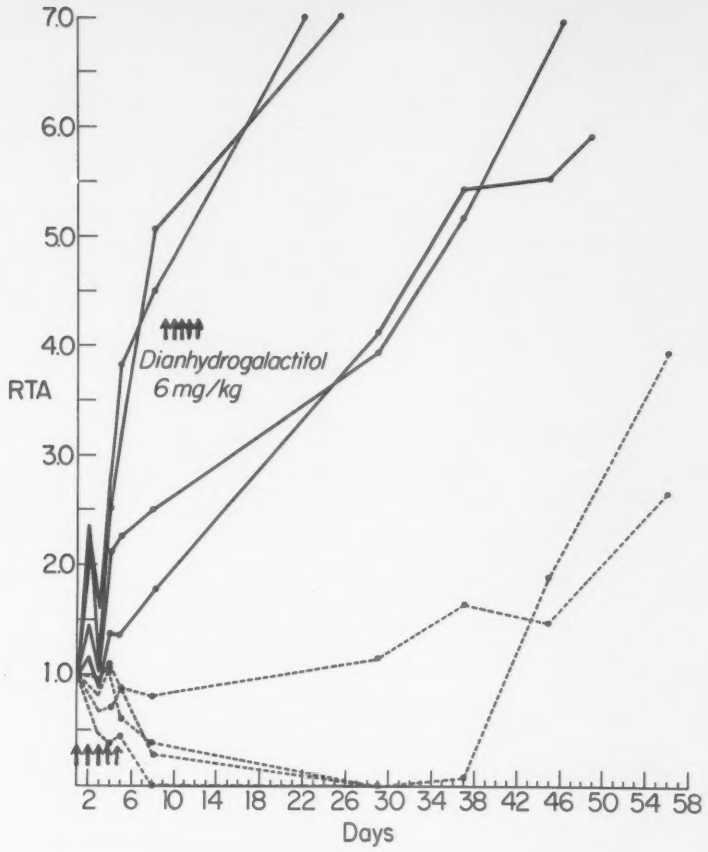

FIGURE 5.—Effect of DAG injected ip on 5 successive days into nude mice bearing human neuroblastoma SK-N-Dz at 6 mg/kg of body weight/day. Solid lines = controls. Dashed lines = treated mice. Each line = 1 mouse. Incomplete tumor lysis was seen with this schedule in some animals. No weight loss or death was observed.

mor lines were derived. These patients were given up to 160 mg/kg of cyclophosphamide without any tumor response observed. The schedule of administration of DAG at the dose range used in this study appears to have less importance in determining its antitumor effect than in determining side effects such as weight loss and animal death. Significant antitumor effects, without accompanying weight loss or deaths, were obtained in mice bearing SK-N-Mc neuroblastoma tumors that were treated with the 5-day schedule (table 3). Similar antitumor effects, with accompanying weight loss and death, were obtained in mice bearing SK-N-Dz tumors that were treated with the 2-day schedule (figs 4 and 5).

In vitro observations of partial cytotoxicity with short duration, single-pulse exposures in asynchronous cell populations and of extensive cytotoxicity with repeated exposures suggest that the DAG-induced antitumor effect may be cell-cycle-dependent. Dependency on the cell cycle for DAG cytotoxicity was demonstrated by Palyi (2), using HeLa cells. Additional support for this comes from the lack of significant additional cytotoxicity after exposure to single-pulsed DAG concentrations > 12 μg/ml (fig 1). Administration of 2-day sequences of DAG to animals appears to be less advantageous than the same total doses spread over a 5-day period. The results of the 5-day DAG treatment sched-

TABLE 4.—In vitro effects of DAG on neural lines derived from patients treated with cyclophosphamide

Cell lines	In vitro effect of DAG*	Highest dose of cyclophosphamide†
Neuroblastoma		
SK-N-Mc	96% cell kill	20 mg/kg/day
SK-N-Lo	7% cell kill	160 mg/kg/day
SK-N-Dz	96% growth inhibition	160 mg/kg/day
SK-N-Sh	75% growth inhibition	40 mg/kg/day
Primitive neuroectodermal tumor		
SK-PN-Ke	68% growth inhibition	100 mg/kg × 2 days
SK-PN-Sc	46% growth inhibition	Previously untreated
SK-PN-Wa	41% growth inhibition	Previously untreated

*See table 1 for in vitro conditions.

†Highest cyclophosphamide doses patients received before viable tumor was obtained for establishing cell cultures.

ule in mice and the greater susceptibility of neuroblastoma tumors than primitive neuroectodermal tumors to the drug suggest that, in the clinical setting, antitumor effects may be anticipated with low doses of DAG over 1 or 2 weeks. Also, clinical resistance to cyclophosphamide in patients with neuroblastoma should not be a contraindication for trials with DAG. Patients with primitive neuroectodermal tumors may, in contrast, have a prior insensitivity to DAG. Assuming that these in vitro and in vivo observations of the effects of DAG in nude mice are predictive for patients, we conclude that the drug warrants continued emphasis in clinical trials in patients with neuroblastoma, with preference given to one or two serial 5-day courses.

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Phase I Study of 3-Deazauridine in the Treatment of Adults With Solid Tumors[1,2,3]

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SUMMARY

A phase I trial of the uridine analog 3-deazauridine was undertaken in 44 adults with solid tumors. The drug was given as a 5-day continuous infusion repeated every 3–4 weeks. The dose-limiting toxic effect was granulocytopenia. Patients with prior nitrosourea therapy or extensive irradiation also had significant thrombocytopenia, and the lowest dose tested, 800 mg/m²/day, was excessive for this group. Mucositis was occasionally severe and was particularly marked in previously irradiated areas. Nausea was mild to moderate. There were isolated episodes of rash, headache, chest pain, and blurred vision. For patients without extensive prior therapy, the recommended dose is 1000 mg/m²/day. No complete or partial remissions were noted.

[Cancer Treat Rep 64:1295–1299, 1980]

The uridine analog 3-deazauridine (DAU) is phosphorylated intracellularly to 3-deazauridine-5-triphosphate, which is a potent competitive inhibitor of cytidine triphosphate synthetase (1). It is marginally active against murine L1210 leukemia but is highly active against L1210 leukemia that is resistant to cytosine arabinoside (2).

In preclinical toxicology studies, male animals tolerated the drug better than females (3). Drug-related toxic effects included emesis, bloody diarrhea, anorexia, renal tubular degeneration with azotemia, hepatic dysfunction, skin ulceration and necrosis, lymphoid depletion, and bone marrow suppression.[6]

Phase I studies at our institution were initially undertaken in adult patients with acute leukemia (3). For solid tumor patients, a starting dose was chosen that was one half the maximum tolerated dose in patients with acute leukemia. The results of our phase I study of DAU administered to adults with solid tumors on a 5-day continuous iv infusion schedule are reported.

PATIENTS AND METHODS

Forty-four patients (31 men and 13 women) with a median age of 51 years (range, 21–73) were treated. Diagnoses included malignant melanoma (nine patients), malignant lymphoma (four), squa-

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[3]Presented in part at the 70th annual meeting of the American Association for Cancer Research, New Orleans, LA, May 17, 1979.

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[6]Castles TR, Sanyer JL, Lee CC, et al. Toxicity of 3-deazauridine (NSC-126849) in mice, dogs, and monkeys. Report No. MR1KC-Pt-3899-74-02. Prepared by the Midwest Research Institute, Pharmacology and Toxicology Division, for the National Cancer Institute, Mar 5, 1975.

mous carcinoma of the lung (two) and of the esophagus (one), oat cell carcinoma of the lung (five), hepatoma (one), transitional cell carcinoma of the bladder (one), leiomyosarcoma (one), glioblastoma multiforme (one), and adenocarcinomas of the lung (eight), colon (two), prostate (two), breast (one), stomach (one), kidney (one), parotid gland (one), and unknown origin (three). Eligibility criteria included demonstrated refractoriness to conventional therapy, granulocyte count ≥ 1500/μl, platelet count ≥ 100,000/μl, BUN ≤ 30 mg/100 ml, serum creatinine ≤ 2.0 mg/100 ml, and serum bilirubin ≤ 2.0 mg/100 ml. With respect to performance status, one patient was asymptomatic (Karnofsky scale, 90–100), five patients were restricted in physically strenuous activity but ambulatory (Karnofsky scale, 70–80), 18 patients were ambulatory and capable of all self-care but unable to carry out any work activities (Karnofsky scale, 50–60), nine patients were capable of only limited self-care and were confined to bed or chair > 50% of waking hours (Karnofsky scale, 30–40), 11 patients were completely disabled and totally confined to bed or chair (Karnofsky scale, 10–20). Eleven patients had CNS involvement. Nineteen patients had previously received either a nitrosourea or extensive radiotherapy. Only one patient had had no prior chemotherapy. Informed consent was obtained from all patients, according to institutional policy.

DAU was supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, as a white crystalline powder in 0.5-g vials. The starting dose of 1500 mg/m²/day was given by continuous infusion in 1000 ml of 5% dextrose in water for 5 consecutive days, and courses were repeated every 3–4 weeks.

A complete history was obtained and physical and appropriate radiologic examinations were performed before the start of treatment. Hemoglobin, wbc count, differential, and platelet count were obtained before each course of treatment and once a week between treatments. Hepatic and renal functions were assessed before each course. Repeated courses of treatment were given after recovery from toxicity, provided there had been no progression of tumor between courses. Dose was escalated in individual patients if there was no toxic effect from the previous course. Response criteria were defined as follows: *complete remission*—complete disappearance of all evidence of tumor; *partial remission*—≥ 50% reduction in tumor size; *stable disease*—< 50% reduction in tumor size and < 25% increase in tumor size for at least 8 weeks; and *progressive disease*—≥ 25% increase in tumor size or the appearance of new lesions.

RESULTS

Responses

Twenty-nine of the 44 patients were evaluable for response after at least one course of therapy. Of these, 13 patients received at least one additional course. Fifteen patients were unevaluable: 11 died < 4 weeks after the start of treatment, one refused to return for followup, two received concurrent treatment with irradiation or surgery, and one was ineligible because of an elevated serum bilirubin. No complete or partial remissions occurred. Three patients had disease stabilization for ≥ 8 weeks, one patient with adenocarcinoma of the lung had a minor response of subcutaneous metastases, and one patient with severe bone pain secondary to metastatic adenocarcinoma of the prostate had complete alleviation of pain but refused to return for objective evaluation or additional treatment. Twenty-four patients had progressive disease with treatment.

Toxicity

One of the 44 patients was ineligible because of an elevated serum bilirubin. The 43 eligible patients received 65 courses of therapy; 53 courses were evaluable for hematologic toxicity. Seven courses were unevaluable due to early death (≤ 21 days after treatment without reaching a clearcut nadir), and five courses were unevaluable due to insufficient blood cell counts.

Patients with extensive prior radiotherapy or prior treatment with a nitrosourea had substantially greater toxic effects than other patients (table 1). At the initial dose of 1500 mg/m², lightly pretreated patients (ie, those who had not previously received a nitrosourea or extensive irradiation) had a median lowest recorded granulocyte count of 200/μl. Despite the degree of myelosuppression, recovery was rapid (< 7 days) and no life-threatening infections occurred in the lightly pretreated group, although three minor infections did occur.

However, three fatal and one nonfatal infection occurred in the heavily pretreated group. Therefore, their starting dose was decreased first to 1000 mg/m² and subsequently to 800 mg/m². This last dose still produced excessive hematologic toxicity in heavily pretreated patients, although it was well-tolerated by others. One heavily pretreated patient with excessive myelosuppression at a dose of 800 mg/m²/day subsequently had tolerable myelosuppression at 600 mg/m²/day. Thrombocytopenia was uncommon in lightly pretreated patients but universal in heavily pretreated patients; it generally preceded granulocytopenia by a few days and, as

TABLE 1.—Lowest granulocyte and platelet counts with DAU by 5-day continuous infusion

Dose (mg/m²/day)	No. of evaluable courses/No. given		Granulocytes (cells × $10^3/\mu l$)			Platelets (cells × $10^3/\mu l$)		
	Initially	Later	Median	Range	Day	Median	Range	Day
			*Heavily pretreated patients**					
1500	8/10	1/1	0.4	0–3.5	12	32	6–97	11
1000	4/5	1/2	0.3	0–1.1	16	29	16–46	15
800	2/2	0/0	0.4	0.1–0.6	19	25	22–28	14
600	0/0	1/1	2.3	—	21	60	—	14
			Lightly pretreated patients					
2500	0/0	1/1	1.1	—	17	303	—	13
2150	1/1	0/0	0.1	—	15	19	—	15
1800	0/0	3/3	0.3	0.3–0.9	25	151	102–155	18
1500	11/13	4/7	0.2	0–4.7	20	123	8–207	15
1000	6/8	4/6	1.8	0.1–6.6	19	204	12–547	15
800	4/4	2/2	2.6	1.0–6.4	21	224	124–263	21

*Patients who had had nitrosourea treatment or radiotherapy.

TABLE 2.—Toxicity of DAU following 5-day continuous infusion

Dose (mg/m²/day)	No. of evaluable courses	Toxic effect (No. of courses)
2500	1	None
2150	1	Nausea (1), diarrhea (1), rash (1)
1800	3	Nausea (2)
1500	24	Nausea (9), mucositis (6), diarrhea (3), malaise (1), chest pain (1)
1000	16	Nausea (4), mucositis (2), diarrhea (1), rash (1), CNS (1)
800	8	Nausea (1), mucositis (1), headache (1)
600	1	None

with granulocytopenia, recovery was usually complete by Day 28 after the start of treatment.

Other toxic effects are outlined in table 2. Fifty-four courses were evaluable. Nausea was minimal. Hepatic and renal dysfunction were not noted. One patient had possible CNS toxicity, as manifested by blurring vision during infusion of the drug. Two patients developed maculopapular rashes thought to be due to the drug. One patient developed chest pain when the rate of drug infusion was increased. The pain disappeared promptly when the rate of infusion was slowed. Mucositis was severe in three patients. In one of these patients it was associated with swelling of the tongue and in the other two it developed in previously irradiated fields.

There were four drug-related deaths; three occurred at 1500 mg/m²/day and one at 1000 mg/m²/day. All four patients had been heavily pretreated with a nitrosourea or extensive irradiation. The three patients treated at 1500 mg/m² had a combination of profound myelosuppression, diarrhea, and septicemia, suggesting that ulceration of the gastrointestinal tract was acting as a portal of entry for bacteria in these neutropenic patients. The patient treated at 1000 mg/m² had received radiotherapy to the oropharynx and developed severe hemorrhagic stomatitis, as well as severe diarrhea. He recovered from the stomatitis but died unexpectedly with persistent myelosuppression on Day 15 of treatment. The cause of death could not be ascertained, and an autopsy was not performed.

DISCUSSION

The major toxic effect of DAU given as a 5-day continuous infusion was granulocytopenia. In patients previously exposed to nitrosoureas or extensive irradiation, thrombocytopenia was also significant. Mucositis was occasionally severe, particularly in previously irradiated fields.

The starting dose in a phase I study is usually one third the lowest dose at which toxicity is seen in the most sensitive large animal species. In a phase I study of DAU previously conducted in leukemia patients (3), myelosuppression and stomatitis were dose-limiting at 3000 mg/m²/day. One half this dose, 1500 mg/m²/day, was chosen as the initial dose in patients with solid tumors in the hope that this would avoid exposing a substantial proportion of the patients to a subtherapeutic dose of the drug. In the first eight evaluable patients, the median granulocyte count nadir was 1600/μl and the median platelet count nadir was 81,000/μl, both of which were within acceptable limits. Based on this, additional patients were entered to confirm the suitability of this dose. However, substantially more toxicity was seen, particularly in heavily pretreated patients who had received nitrosoureas or radiotherapy. At a 50% lower dose, 1000 mg/m²/day, toxicity was still excessive in heavily pretreated patients but acceptable in others. Even with an additional 20% decrease in dose to 800 mg/m²/day, heavily pretreated patients experienced excessive myelosuppression, and since little antitumor activity had been seen in patients treated at almost twice this dose, it was felt that it was not justifiable to continue the study. Hence, we cannot recommend a suitable starting dose for heavily pretreated patients, but other patients receiving a 5-day continuous infusion of DAU should be treated initially at a dose of 1000 mg/m²/day every 4 weeks. The dose may be escalated by 20% in subsequent courses if, in the first course, the lowest recorded granulocyte and platelet counts are ≥ 1500/μl and 100,000/μl, respectively, and if no mucositis or diarrhea occurs. This study indicates that it is difficult to use data from a phase I study of leukemia patients in order to choose an appropriate dose for solid tumor patients.

Overall, 11 patients died within 4 weeks of the

start of treatment. Of these, the four patients with drug-related deaths were considered evaluable for toxicity, leaving seven of 43 patients (16%) unevaluable for toxicity because of early death. All seven of these patients were completely bedridden and disabled before entry into the study and were recognized as not fulfilling the usual criterion for a phase I study: a life expectancy of ≥ 12 weeks. Patients with poor performance status were included in the study for two reasons. First, the continuous infusion schedule being followed required hospitalization, and these patients were already hospitalized. Second, a number of patients with poor prognosis because of brain metastases were entered since it was demonstrated that DAU penetrates readily into the human CNS (4). If patients with a pretreatment life expectancy of ≤ 12 weeks were excluded from analysis, the conclusions regarding type of toxicity and recommended dose would not be altered. Therefore, we feel that this study gives a valid qualitative and quantitative estimation of the toxicity of DAU despite the high early death rate.

Although little therapeutic benefit was seen in this phase I study, further testing would be necessary to accurately define the antitumor activity of DAU against solid tumors. In addition, DAU may potentiate the effect of other antimetabolites; eg, by lowering intracellular cytidine triphosphate levels, it could potentiate the activity of cytosine arabinoside (5). It may deserve additional investigation as a treatment of primary and metastatic tumors of the brain since it readily penetrates cerebrospinal fluid, brain, and intracerebral tumors (4).

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Phase I Trial of PALA[1,2]

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SUMMARY

PALA was given iv on a weekly schedule to 32 patients with advanced malignant tumors. A course of treatment consisted of three weekly doses. Patients were treated at eight dose levels, ranging from 900 to 6750 mg/m². Gastrointestinal toxicity (diarrhea) and skin rash were dose-limiting. No consistent myelosuppression occurred, and no renal or hepatic toxicity was observed. Two patients who did not have metastatic intracranial disease experienced episodes of encephalopathy and seizures. No major therapeutic responses were observed during this phase I trial; however, minor responses (≥ 25% tumor decrease) occurred in three patients (one with adenocarcinoma of the lung and two with epidermoid carcinoma of the bladder). Doses of 4500 mg/m²/week for patients with a performance status ≥ 70 and 3750 mg/m²/week for patients with a lower performance status are recommended for phase II studies.

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PALA is an inhibitor of de novo pyrimidine biosynthesis and has a mechanism of action which differs from that of currently used agents. It is an analog of the transition state in the reaction catalyzed by aspartate transcarbamoylase, binding to the enzyme about 1000 times more tightly than carbamoyl phosphate, the physiologic substrate most tightly bound (1). PALA blocks the growth of mammalian cells in culture (2) and in vivo (3). Reversal of this growth inhibition can be accomplished in vitro by the addition of uridine to the culture medium (2). Uridine prevents both the toxic and antitumor effects of PALA in mice bearing Lewis lung carcinoma, and carbamoyl-DL-aspartate reverses the antitumor effects (4). This reversal of PALA activity in vivo by carbamoyl-DL-aspartate supports the concept that the drug's antitumor activity is due to specific inhibition of aspartate transcarbamoylase.

In experimental tumor models, PALA is active in several solid tumor systems including Lewis lung carcinoma, B16 melanoma, glioma 26 (5,6), and colon 26 (7). Unlike other antimetabolites, it has been observed to have low activity against murine leukemias (L1210 and P388) (5,6). In addition, PALA has antitumor activity in the carcinogen-induced FANFT bladder carcinoma (8). Johnson et al (5) proposed that sensitivity to PALA is related to intracellular concentrations of aspartate transcarbamoylase. They demonstrated that the rapidly growing, PALA-resistant murine leukemias have higher levels of the enzyme than the more slowly growing, PALA-sensitive, experimental solid tumors (5).

In toxicologic studies the most prominent findings in dogs and monkeys were gastrointestinal and CNS abnormalities. These were manifested by anorexia, weight loss, diarrhea, bloody rectal discharge, sedation, muscle tremors, and convulsions. Electroencephalograms were within normal limits. Hematologic effects were minor, with leukopenia in monkeys

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and leukocytosis in dogs. Cutaneous toxic effects of peeling skin and alopecia were noted in monkeys.[5] A single-dose administration schedule proved to be less toxic than a multiple-dose regimen (daily × 5) in dogs; 2400 mg/m² was the lowest toxic dose.[5]

In murine tumors, a single dose of PALA resulted in > 90% inhibition of aspartate transcarbamoylase activity 24 hours after administration and persistence of an 80% inhibition 6 days after the drug dose (9). This study reports the results of a phase I trial of PALA given as a single dose every 7 days.

MATERIALS AND METHODS

Eligibility requirements for patients entered in this trial included: performance status ≥ 50 (Karnofsky scale), histologic evidence of malignancy in patients for whom standard therapy was not indicated, and an estimated survival of at least 8 weeks. Patients had not received chemotherapy or radiation therapy for at least 4 weeks before receiving PALA. Acceptable hematologic status (wbc count ≥ 4000/mm³ and platelet count ≥ 100,000/mm³), with adequate hepatic function (bilirubin ≤ 1.4 mg/dl) and renal function (creatinine ≤ 1.7 mg/dl), were required in all patients.

PALA was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), in sterile vials containing 1 g dissolved in 10 ml of sterile water for injection buffered with sodium hydroxide to adjust to pH 6.5–7.5. The dose of PALA was added to 50 ml of normal saline immediately before administration. The drug was given by iv infusion over a period of 30 minutes each week for 3 weeks. Courses were repeated every 8 weeks in the absence of drug-related toxic effects. The initial dose level was 900 mg/m²/week, with escalations carried out according to a modified Fibonacci search scheme (10) (table 1). At least three patients were entered at each dose level.

Pretreatment evaluation of patients included a complete history and physical examination, with laboratory evaluation consisting of cbc, platelet, and differential counts, 12-channel biochemical profile, plasma creatinine, serum electrolytes and glucose, 5′-nucleotidase, prothrombin time, urinalysis, ECG, and chest roentgenogram. Followup studies included all of these tests, except for ECG and chest roentgenogram, repeated three times per week initially and weekly thereafter.

Thirty patients who received PALA were evaluable for manifestations of toxicity. The median age of the 19 men and 11 women was 56 years (range, 34–73), and the median performance status was 60 (range, 50–90); 14 patients had a performance status ≥ 70. Prior therapy included chemotherapy in eight patients, radiation therapy in three, and both treatments in 18. The types of tumors in these patients are listed in table 2.

Although measurable disease was not required for inclusion in this phase I trial, an evaluation of therapeutic efficacy was made in those patients with objectively measurable disease parameters. Response criteria were defined as: *partial remission*—a > 50% decrease of all measurable disease for at least 1 month; and *minor response*—an objective tumor decrease ≥ 25% but less than that required for a partial remission.

RESULTS

Thirty of 32 patients with advanced malignancies who were entered in this phase I study received adequate and evaluable trials; they received a median of one 3-week course (range, one to three). Two patients who had inadequate courses died of progressive disease without evidence of drug-related toxic effects, after having received only one dose of PALA.

[5]Clinical brochure: PALA. Prepared by the National Cancer Institute, Bethesda, MD, Dec 1977.

TABLE 1.—Phase I study of PALA: hematologic toxicity

Dose (mg/m²/wk)	No. of patients entered	No. of evaluable patients	Wbc count nadir (cells × 10³/mm³)		Platelet count nadir (cells × 10³/mm³)	
			Median	Range	Median	Range
900	4	3	4.5	4.4–4.6	134	72–220
1300	3	3	4.4	3.4–7.5	148	130–299
2000	3	3	4.4	4.3–4.7	200	188–225
3000	3	3	6.2	4.4–8.1	220	160–350
3750	5	5	4.1	1.8–8.6	153	58–450
4500	12	11	5.7	3.0–7.8	212	100–330
5600	7	7	5.6	2.0–7.8	302	55–354
6750	3	3	4.4	4.3–13.1	219	120–555

TABLE 2.—Phase I trial of PALA: therapeutic responses

Tumor type	No. of patients	No. of— Partial remissions	No. of— Minor responses
Bronchogenic carcinoma	13*	0	1
Bladder carcinoma	7	0	2
Renal cell carcinoma	3	0	0
Head and neck carcinoma	2	0	0
Unknown primary adenocarcinoma	2	0	0
Sarcoma	2	0	0
Lymphocytic lymphoma	1	0	0
Totals	30	0	3

*10 patients with adenocarcinoma and 3 with epidermoid carcinoma.

The dosage levels investigated, the number of patients entered at each level, and the hematologic effects of PALA are shown in table 1. Fewer than 15% of the patients experienced any myelosuppression; these cases did not appear to be dose-related. There were no episodes of drug-induced anemia; the minor myelosuppression did not lead to infection or hemorrhage in any patient. All of the four patients who showed a decrease in wbc or platelet counts had been previously treated with chemotherapy, with two having received radiation therapy as well. There was no consistent pattern in the timing of the blood cell count decreases; however, the blood cell count nadirs generally appeared within the first 2 weeks of starting PALA, and recovery to normal levels occurred within 10 days. There was no evidence of any cumulative myelosuppression, and at the highest dose level, no patient showed a decrease in blood cell counts below the normal range.

The most prominent toxic manifestation was skin rash. This generally appeared first as an erythematous macular eruption, which became confluent and scaling, leading to areas of denudation and skin ulceration. The rash initially involved the face, scalp, and intertriginous areas, later progressing to involve the trunk and extremities. Biopsy of these lesions revealed perivascular inflammation, with vessel disruption and epidermal abnormalities including edema and loss of the keratin layer. Advanced lesions showed more severe involvement of the epidermis, with only a single cell layer remaining and with infiltration of the dermis by lymphocytes. Skin toxic effects were graded as follows: 0 = no skin abnormality; 1+ = rash involving < 10% of body surface area; 2+ = involvement of 10%–25% of body surface area; 3+ = rash involving 25%–50% of body surface area; and 4+ = involvement of > 50% of body surface area or the onset of erythema multiforme, which was observed in two patients. The occurrence and severity of skin toxicity was dose-related (table 3). The rash appeared as early as Day 5 of treatment but occasionally was not observed until after the second weekly dose of PALA (Day 10). Clearing of the toxic effects generally required 1–2 weeks without PALA, from the onset of the skin rash.

As predicted by large animal toxicity studies, gastrointestinal toxicity was a frequent finding in this phase I trial. This was generally manifested by diarrhea, which had a timing of onset similar to that of the skin toxicity. The earliest toxic effects were observed on Day 5 or 6, but diarrhea did not occur in some patients until the second week of treatment. Criteria for grading the severity of the gastrointestinal toxic effects were established as follows: 0 = no gastrointestinal symptoms; 1+ = mild, watery diarrhea (fewer than five bowel movements per day) which did not interfere with normal activities; 2+ = uncomfortable diarrhea (with five or more bowel movements per day) which interfered with normal activities; 3+ = profuse, painful, bloody diarrhea; and 4+ = diarrhea with fluid and electrolyte imbalance requiring hospitalization. The number of patients who experienced gastrointestinal toxic effects and the severity of these effects are shown in table 3. The diarrhea was dose-related and was similar to the skin toxic effects in its severity at various dose levels. The diarrhea cleared within 1 week of its onset in all cases.

Sigmoidoscopy was performed in eight patients who experienced gastrointestinal toxic effects; findings were identical in all patients. Friability and granularity of the bowel mucosa were observed; there were ulcerations in patients with ≥ 2+ toxicity. The appearance of these lesions on colonoscopy was indistinguishable from the findings of ulcerative colitis. In addition, biopsy of the lesions showed a histologic picture consistent with ulcera-

TABLE 3.—Phase I study of PALA: cutaneous and gastrointestinal toxicity (30 patients)

Dose (mg/m²/wk)	No. of evaluable patients	No. of patients				
		Toxicity level—				
		0	1+	2+	3+	4+
		Cutaneous				
900	3	3				
1300	3	3				
2000	3	3				
3000	3	2	1			
3750	5		2	2		1
4500	11	1	5	2	3	
5600	7		1	4	1	1
6750	3				1	2
		Gastrointestinal				
900	3	3				
1300	3	3				
2000	3	2	1			
3000	3	3				
3750	5	1	3	1		
4500	11	1	6	2	1	1
5600	7		1	4	2	
6750	3			1	1	1

tive colitis; there was a decrease in the number of microvilli, and inflammatory cells were present in the lamina propria. The abnormalities were restricted to the mucosa and submucosa. Repeat sigmoidoscopy showed that all abnormalities had cleared within 4–10 days after the onset of symptoms.

Stomatitis occurred frequently but was generally less severe than the skin or gastrointestinal toxic effects; it was also observed within the first 2 weeks of treatment and resolved within 4–7 days. Acute toxic effects were generally mild, but during the drug infusion a sensation of facial tingling was noted by one half of the patients at the three highest dose levels. This sensation was eliminated or lessened by slowing the infusion rate. Nausea, without vomiting, occurred only at the three highest dose levels. It occurred in only one third of the patients, was mild, and did not require treatment with antiemetics. No hepatic or renal toxicity was observed during this phase I study.

As previously noted, CNS toxicity was prominent in preclinical toxicity studies. In this phase I trial, two patients exhibited neurologic abnormalities possibly related to PALA. A 73-year-old man with epidermoid carcinoma of the bladder experienced two seizures followed by persistent encephalopathy 1 week after his second dose of PALA at a dose of 4500 mg/m². CT did not reveal presence of metastasis, but there was evidence of a previous cerebrovascular accident. The patient had no further seizures; however, he remained encephalopathic for several weeks after chemotherapy was discontinued. A 49-year-old woman with sarcoma also experienced seizures. She had undergone neurosurgery for placement of a thalamic electrode due to intractable pain 3 years before receiving PALA. During that period she had had no neurologic complications. After her third dose of PALA, she gradually developed confusion and lethargy, followed by several generalized seizures. Evaluation for structural lesions in the CNS did not reveal abnormalities other than the presence of the intracranial electrode. The encephalopathy cleared over a 2-week period, and the patient, who is not receiving PALA or anticonvulsive medications, has had no further seizures or neurologic symptoms. Electroencephalograms in six patients before and after PALA did not reveal any specific abnormalities.

Antitumor activity was observed in three of the 30 adequately treated patients. Three of the 13 patients with bronchogenic carcinoma who were treated at ≥ 3750 mg/m² had measurable disease and will be included in a subsequent phase II trial. Only minor responses occurred (table 2). These included a 40% decrease of a right upper lobe mass and a mediastinal lesion lasting for 2 months in a 66-year-old man with adenocarcinoma of the lung and a 25% decrease in bilateral pulmonary metastases lasting for 1 month in each of two patients with epidermoid carcinoma of the bladder. No partial remissions were observed.

DISCUSSION

In this phase I trial using PALA iv on a weekly schedule, dose-limiting toxic effects were skin rash

and diarrhea. Gastrointestinal effects of PALA were predicted in preclinical large animal studies; however, cutaneous toxic effects were noted only in monkeys and were not as prominent as those observed in this phase I trial. Two patients with advanced malignancies developed CNS abnormalities consisting of encephalopathy and seizures, which cannot definitely be attributed to PALA. However, in the absence of brain metastases or past history of symptoms, it appears likely that PALA was the cause of the neurologic abnormalities, which were similar to the toxic effects seen in large animal studies. Additional close neurologic monitoring will be necessary during phase II studies, especially in those patients with intracranial abnormalities.

Unlike currently used inhibitors of pyrimidine biosynthesis, PALA showed no consistent hematologic toxicity. Fewer than 15% of patients experienced myelosuppression; this was not dose-related and occurred in heavily pretreated patients.

A correlation between PALA sensitivity and low tissue aspartate transcarbamoylase activity has been proposed in experimental tumor systems (5). With the demonstration of high aspartate transcarbamoylase levels in mammalian tissues with high mitotic activity, such as intestinal mucosa (11), and with the observed toxicity in this study, it appears that sensitivity of normal cells to PALA is not dependent on the enzyme concentration alone. Additional factors such as availability of uridine and tissue levels of PALA probably play a role in the toxicity of the drug.

Patients who were entered in this phase I trial were generally of good performance status and were able to tolerate PALA well on an outpatient basis. Although patients with a poorer performance status were not predisposed to greater toxic effects, they had more difficulty tolerating drug-related side effects. For phase II studies using PALA as a single agent in a weekly schedule, we recommend an initial dose of 4500 mg/m^2 iv in patients with a performance status $\geq$ 70. In patients with a lower performance status, an initial weekly dose of 3750 mg/m^2 is recommended.

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Thymidine Arrest and Synchrony of Cellular Growth In Vivo[1,2]

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SUMMARY

Thymidine (TdR) has been used to study the kinetics of in vitro cell proliferation and is currently being used clinically as a single agent at high doses. We have explored the in vivo cytokinetic effects of TdR on rapidly proliferating cell populations by continuous infusions in rats. The nucleoside was lethal at high doses when serum levels approached 10^{-2} M. At levels of 10^{-3} M, TdR exposure for > 24 hours resulted in bone marrow hypocellularity and peripheral myelosuppression. Pathologic findings were also noted in the intestinal mucosa. Serum TdR levels of 10^{-4} M were sufficient to induce arrest of cell growth in S phase by inhibition of DNA synthesis. Subsequent release after TdR exposure produced partial synchronization of the bone marrow and intestinal mucosa cell populations, as shown by microfluorometry and labeling studies to monitor DNA synthesis. A similar arrest of cell cycle traverse has been demonstrated in a tumor cell population by infusing rats bearing transplantable subcutaneous myeloblastomas. The inhibition of DNA synthesis, as determined by labeling studies, was comparable for bone marrow, intestinal mucosa, and myeloblastoma at serum TdR levels of 10^{-3} M. This arrest of myeloblast cell growth was dependent on tumor burden and did not effect survival when maintained during 72-hour infusions. The continuous infusion of TdR provides an approach for studying cell kinetics in vivo, and findings similar to those described here have been observed in our clinical studies.

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Synchronization of mammalian cell growth has been studied following exposure of tissue cultures to high concentrations of thymidine (TdR) (1–8). Inhibition of the cell cycle occurs at TdR concentrations of 10^{-4} to 10^{-3} M and results in the accumulation of cells in early S phase (5,6). Growth inhibition involves feedback regulation of several enzymes in pyrimidine biosynthesis (6). These include thymidine kinase, deoxycytidine monophosphate deaminase, and cytidine diphosphate reductase (2,6). The mechanism responsible for blocking DNA synthesis acts primarily by inhibiting conversion of cytidine 5′-diphosphate to deoxycytidine 5′-diphosphate, with a resultant deficiency of deoxycytidine 5′-triphosphate (9–15). This deficiency is clearly critical in that the addition of deoxycytidine to culture medium results in a bypass of the inhibition of cytidine 5′-diphosphate reductase and release of the block (12).

TdR inhibition of DNA synthesis results in synchrony by blocking cells in S phase while allowing

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remaining cells in other phases to continue cell cycle traverse and accumulate in G_1-early S (6). Following removal of TdR blockade, the cell population proceeds in a synchronous manner through S phase and mitosis (6,12). Synchronized cells afford an opportunity to study metabolic events in the cell cycle as well as the cytokinetic specificity of chemotherapeutic agents.

TdR has also been shown to have cytotoxic properties. Exposure of HeLa cells to 2 mM TdR inhibited DNA synthesis and resulted in synchronization of growth after removal of the nucleoside (2). Cell viability was reduced when cells were exposed to this concentration of TdR for periods greater than one generation time. Enhanced cytotoxicity was observed by prolonging the duration of exposure and increasing the TdR concentration (2). Excess TdR has also been shown to cause chromosomal aberrations in Chinese hamster cells (16).

Recent studies have shown that TdR may have selective antitumor toxicity. Human melanoma and colon carcinoma lines showed a greater in vitro sensitivity to the lethal effects of TdR exposure (1 mg/ml for 72 hours) than non-neoplastic control lines (17); < 23% of the tumor cells survived 72 hours, compared with > 60% of control cells. Colony formation was equally affected, with only 4.3% of treated tumor cells forming colonies, in contrast to 33% of the nontumor cells. These studies were extended to an in vivo system using human melanoma transplanted into nude mice (18). TdR was administered at a dose of 1 g/kg/hr ip for at least 72 hours. Excess TdR specifically suppressed the growth of human melanoma for the duration of the treatment with no apparent host toxicity. The difference in tumor volume between treated and untreated animals was significant at the $P < 0.01$ level. Similar in vivo antitumor effects due to TdR have been confirmed in other human tumor xenograft models (19,20).

The cytokinetic effects of TdR have not been thoroughly explored in vivo. This paper reports a study of the effects of continuous infusion in an animal model. Cell cycle traverse arrest of rapidly proliferating cell populations (bone marrow, intestine, and tumor) are correlated with TdR dose rates and resultant blood levels. Cellular kinetics of the partially synchronized populations are also determined by monitoring the rate of DNA synthesis or cellular growth after the removal of excess TdR. These results support the conclusion that TdR can be used to study and modulate the kinetics of proliferating cells in vivo. This approach may be applicable in altering the sensitivity of cell populations to chemotherapeutic agents.

MATERIALS AND METHODS

TdR infusions.—A technique has been recently described for the continuous iv infusion of chemotherapeutic agents in a characterized, inbred rat model of acute myelogenous leukemia (21–23). The methodology has been adapted for TdR infusions in normal Wistar/Furth rats weighing 175–200 g. These animals were obtained from Microbiological Associates, Bethesda, MD. Animals were housed two per cage and fed standard laboratory chow. TdR was obtained from Sigma Chemical Co, St. Louis, MO. Solutions were prepared at a TdR concentration of 50 mg/ml in 0.3% (wt/vol) sodium chloride. The solutions were filtered for sterility and stored at room temperature. Drug doses were calculated on the basis of body weight. Infusions were performed with a Harvard infusion pump, model 940, with an adapter to hold eight syringes (Harvard Apparatus, Inc). Those infusions requiring volumes beyond the capacity of the Harvard pump were performed with IMED volumetric 922 infusion pumps (IMED Corp, San Diego, CA).

TdR assays.—TdR levels were measured in serum samples by high-pressure liquid chromatography, as described previously (24).

Cell kinetic studies.—Rat bone marrow cells were obtained by flushing excised femurs with cold Dulbecco's phosphate-buffered saline (PBS) without calcium. Samples were spun at 1000 × *g* for 7 minutes at 4°C and the pellets were resuspended in 7.0 ml of PBS. Bone marrow cell counts were performed in duplicate in a hemocytometer. Aliquots of 0.25 ml were added to 4.75 ml of 0.05 mg/ml of propidium iodide monohydrate (Calbiochem, San Diego, CA) in 1.1% sodium citrate for flow microfluorometric analysis, according to the method of Krishan (25). After 30 minutes of staining, samples were analyzed for DNA content per cell on a cytofluorograf, model 4801 (Biophysics Systems, Mahopac, NY). DNA distribution histograms were analyzed using a computer-assisted program designed to quantitate the percentage of cells with G_0-G_1, S, and G_2-M DNA content. The in vivo labeling studies were performed by ip injection of 250 microcuries of ^{32}P, specific activity 9.1 curies/mmol (New England Nuclear Corp, Boston, MA). After appropriate time intervals, the bone marrow cells were prepared from an excised femur as described above. Intestinal mucosa cells were obtained from the proximal 2 cm of small intestine after gentle separation from the muscularis layer. Subcutaneous myeloblastomas were excised and separated from connective tissue. The tumor was finely minced and prepared as a single cell suspension by aspirating gently through a Pasteur pipette. Cell preparations

TABLE 1.—Effect of TdR infusion on serum level and bone marrow cell counts*

Infusion	TdR level (10^{-4} M)	Bone marrow cell count ($\times 10^6$/mm^3)
Saline		
24 hrs	ND†	12.6 ± 2.3
48 hrs	ND	11.5 ± 0.1
72 hrs	ND	13.3 ± 2.4
TdR		
50 mg/kg/hr		
24 hrs	0.79 ± 0.09	7.3 ± 1.8
48 hrs	0.88 ± 0.01	14.0 ± 0.8
72 hrs	1.06 ± 0.25	8.2 ± 2.9
100 mg/kg/hr		
24 hrs	1.60 ± 0.30	7.2 ± 0.3
48 hrs	1.20 ± 0.20	8.1 ± 2.1
72 hrs	1.70 ± 0.10	7.6 ± 0.6
200 mg/kg/hr		
24 hrs	2.80 ± 0.10	6.7 ± 0.4
48 hrs	3.20 ± 0.20	7.3 ± 1.2
72 hrs	3.60 ± 0.40	5.6 ± 1.4
400 mg/kg/hr		
24 hrs	3.50 ± 2.00	7.6 ± 1.1
48 hrs	5.10 ± 0.30	6.6 ± 0.1
72 hrs	5.90 ± 0.10	3.9 ± 0.6
600 mg/kg/hr		
24 hrs	4.80 ± 0.30	7.0 ± 0.6
48 hrs	11.90 ± 2.10	5.1 ± 1.0
72 hrs	9.00 ± 0.30	3.5 ± 1.3
800 mg/kg/hr		
24 hrs	87‡	4.4
48 hrs	102§	4.8
1000 mg/kg/hr		
12 hrs	500§	—

*Wistar/Furth rats (175–200 g) were given TdR by continuous infusion through the jugular vein as described in ref 21. Serum TdR levels = mean values ± SD obtained for 2 rats on completion of the infusion. Then, bone marrow cells were obtained from an excised femur from each rat. Values = mean ± SD of determinations from 2 animals after infusion, except that values = a single determination at 800 and 1000 mg/kg/hr.

†ND = not detectable.

‡1 animal survived.

§Both animals died.

were spun at 1000 × *g* for 7 minutes at 4°C and washed twice in 7 ml of PBS. The pellets were resuspended in PBS at a final concentration of 1×10^6 cells/ml. Alkali digestion was carried out by adding 0.5 ml of 0.6 N NaOH to 2 ml of cell suspension and incubating overnight at 37°C. Aliquots were then precipitated with 50% trichloroacetic acid (TCA), collected on Whatman GF/C filters and washed with 5% TCA. The filters were washed with absolute alcohol, dried, and placed in scintillation vials containing 5 ml of Omnifluor (New England Nuclear Corp). Vials were counted in a Beckman model LS-335 liquid scintillation system.

RESULTS

TdR infusions.—The various TdR dose rates given in the rat by continuous infusion over 24, 48, and 72 hours are shown in table 1. The dose rate was escalated from 50 to 1000 mg/kg/hr. At the lower dose rate, serum TdR approached 10^{-4} M, while at a higher dose rate of 600 mg/kg/hr, the serum levels reached 10^{-3} M. The TdR serum levels obtained after infusions of 24, 48, and 72 hours at the various dose rates are shown in figure 1. The three curves are similar in appearance, with an initial rise to a dose of 200 mg/kg/hr and then a more

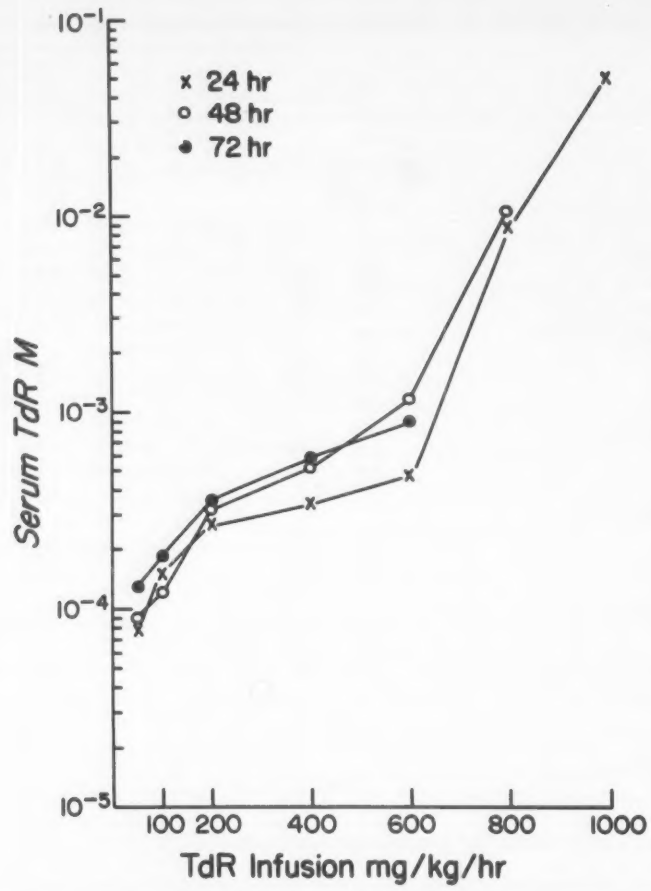

FIGURE 1.—Rats were infused with TdR at 50–100 mg/kg/hr. Steady-state serum levels were monitored after infusions of 24 (x), 48 (○), and 72 (●) hrs (table 1). Lethal effects were dependent on duration at doses > 600 mg/kg/hr.

gradual slope through a dose of 600 mg/kg/hr. At doses > 600 mg/kg/hr, the TdR level again rose sharply, as if overriding a clearance mechanism. Lethal effects occurred at these higher dose rates; half of the animals died after 24 hours and the rest died after 48 hours at 800 mg/kg/hr. Appropriate controls given similar volumes of saline tolerated these infusions without evidence of toxicity due to salt loading.

Effect of TdR on bone marrow cellularity.—Bone marrow cell counts were performed on rat femurs on completion of the infusion. These preparations included terminally differentiated cells and were not subjected to Ficoll-Hypaque separation. The cell counts listed in table 1 represent the mean value obtained for two rats infused at each dose rate and duration. Analysis of the data by linear regression indicated that the decline in all cell counts with increasing dose rates is significant at a level of $P < 0.001$. A nonparametric linear regression of the cell counts for 24-hour infusions at the different doses showed no significant effect at a significance level of $P < 0.005$. The effect of TdR exposure on bone marrow was, therefore, more pronounced with the longer infusions at higher dose rates.

Effect of TdR on peripheral blood cell counts.—

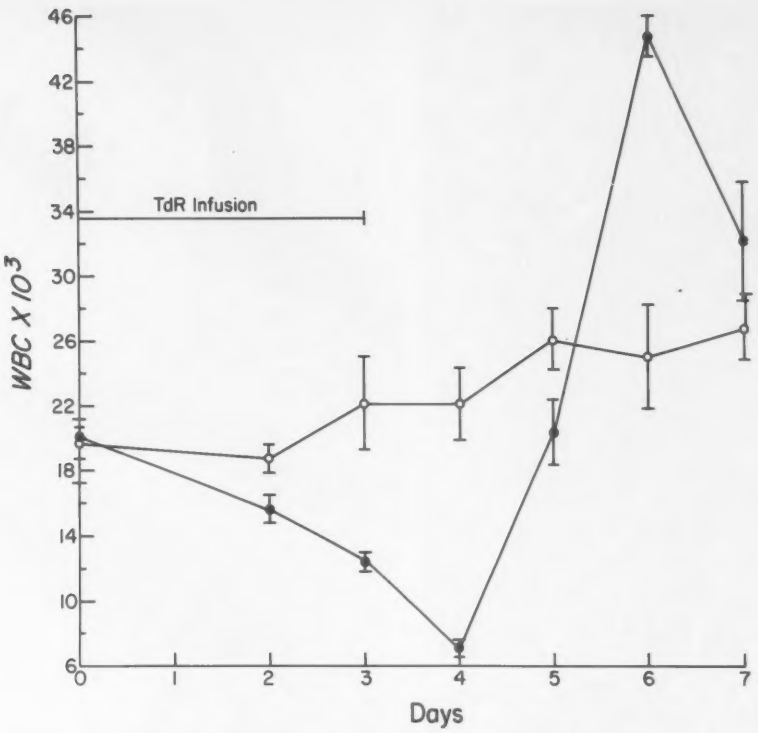

FIGURE 2.—Rats (175–200 g) were infused with TdR at doses of 200 (○) and 600 (●) mg/kg/hr for 72 hrs. Peripheral blood leukocytes were monitored by tail vein bleeding and counting in a hemocytometer.

Peripheral blood leukocytes were also monitored to determine the cytokinetic effects of TdR at doses of 200 and 600 mg/kg/hr for 24, 48, and 72 hours. Baseline leukocyte counts were obtained before treatment, during the TdR infusion, and each day thereafter for 14 days. Infusions at either 200 or 600 mg/kg/hr for 24 and 48 hours failed to produce a > 25% decline in leukocyte count (data not shown). Figure 2, however, compares the leukocyte counts of rats infused for 72 hours at both of these doses. Animals receiving infusions at 600 mg/kg/hr had a progressive decline in leukocyte counts during the period of infusion and for 24 hours thereafter. A rapid recovery was noted by Day 5, followed by a marked rebound above baseline levels on Days 6 and 7. This finding is in contrast to those values obtained in animals infused at 200 mg/kg/hr for the same time period. Under these circumstances, there is no significant decline in peripheral blood cell counts.

Pathologic changes.—The subsequent decline in leukocyte counts was correlated with pathologic changes in the bone marrow sections. As shown in figure 3A, a preparation of normal rat bone marrow shows adequate cellularity, with erythroid and myeloid elements that appear to be normal in contrast to the rat bone marrow preparation shown in figure 3B, from an animal given a TdR infusion of 600 mg/kg/hr for 72 hours. Moderate hypocellularity is shown in this representative specimen, and similar findings have been consistently observed in bone marrow of other rats infused with TdR. Megaloblastic changes have also been observed in these bone marrow sections. Pathologic examination of specimens from other organs (lung, gastrointestinal tract, heart, liver, and kidney) revealed changes only in the intestinal epithelium. The intestinal epithelium from a rat infused with saline (fig 3C) is included for comparison with that prepared from another rat infused with TdR at 600 mg/kg/hr for

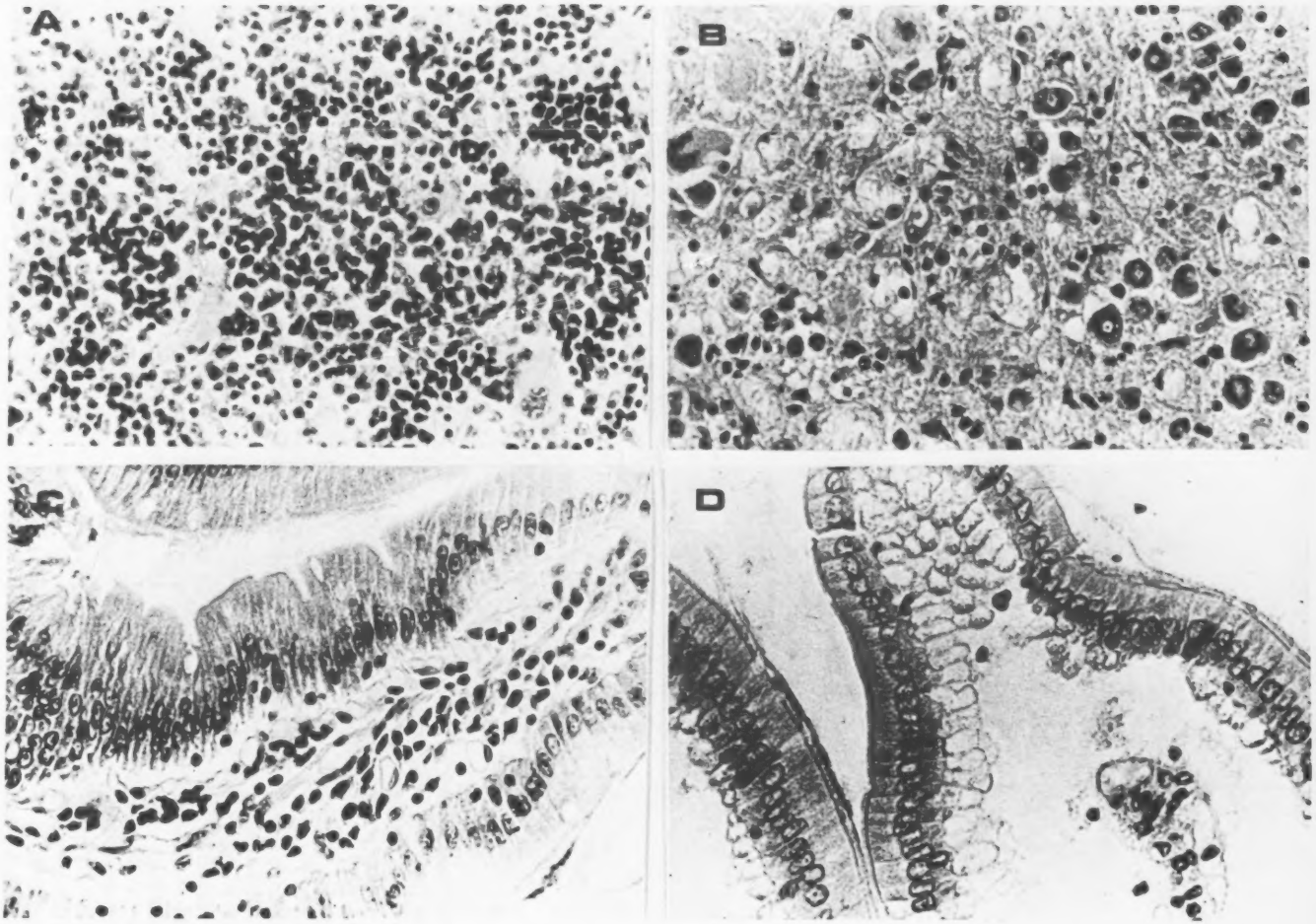

FIGURE 3.—Photomicrographs of bone marrow (A) and intestine (C) from a rat infused with saline for 72 hrs. Similar preparations of bone marrow (B) and intestine (D) from a rat infused with TdR at 600 mg/kg/hr for 72 hrs. After exposure to TdR, the bone marrow exhibits hypocellularity. Intestine from the treated rat shows edema of the lamina propria.

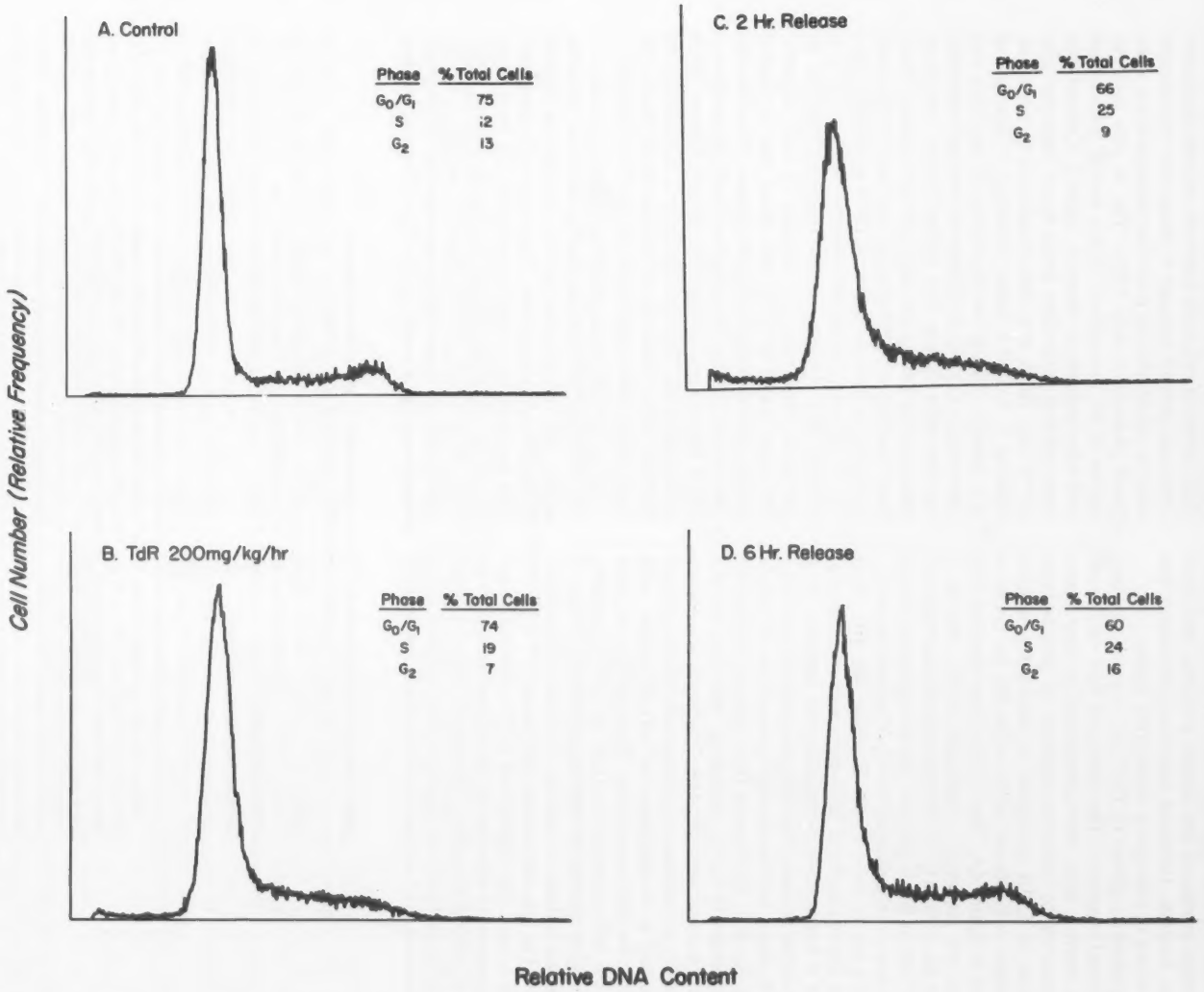

FIGURE 4.—Bone marrow DNA histograms from rats infused with saline (A) for 24 hrs and TdR (B) at 200 mg/kg/hr for 24 hrs. DNA histograms were performed on rats infused with TdR at 200 mg/kg/hr for 24 hrs and at 2 hrs (C) and 6 hrs (D) after completing the infusion. Results are expressed as mean ± SE for 3 animals in each group.

72 hours. This preparation (fig 3D) showed a characteristic edema of the lamina propria, with lymphatic dilation. Denuded intestinal epithelium was also noted in rats given lethal TdR doses.

Cytokinetic effects of TdR on bone marrow and intestinal mucosa.—The effects of TdR on the rat bone marrow cells were monitored by flow microfluorometry to detect alterations in proliferation kinetics. A DNA histogram of bone marrow from a control rat infused with saline for 24 hours is shown in figure 4A. The G_0-G_1 phase contains 75% of the cell population, with 12% of the cells in S phase and 13% in G_2-M. Following a 24-hour infusion with TdR at 200 mg/kg/hr, the histogram is altered with less prominence of the G_2-M phase and an accumulation of cells in the S phase (fig 4B). Rats given 200 mg/kg/hr of TdR were also monitored at 2 and 6 hours after completing the infusion. At 2 hours after release, the cells progressed from G_0-G_1 into S phase with a constant proportion of cells in G_2-M (fig 4C). At 6 hours after release, 60% of the cells remain in G_0-G_1, compared with 75% for the control marrow cells (fig 4D). This decline is accompanied by an increase in S phase to twice the control value and a re-emergence of a G_2-M phase, with 16% of the cell population. This pattern of bone marrow cells arrested in G_1-S by TdR exposure, followed by partial synchronous growth through S phase and mitosis on release, has been consistently observed in rats with serum TdR levels $> 1 \times 10^{-4}$ M for 24 hours. The half-life of TdR in the rat is approximately 45 minutes at plasma levels of 10^{-4} to 10^{-3} M (data not shown). Therefore, in vivo release and cell cycle traverse might be more uniform under circumstances of a more rapid decline in serum TdR or, for example, of rescue with deoxycytidine (12). TdR arrest of cell cycle traverse and resultant synchronous growth through S phase after release can also be monitored by following incorporation of labeled precursors into DNA. The in vivo incorporation of ^{32}P into cellular DNA during TdR arrest and after release is shown in table 2. Control rats were infused with saline for 24 hours. Incorporation of ^{32}P into DNA was then monitored in three animals at 2, 4, and 6 hours following ip injection of the isotope. Linear incorporation of the label was observed in the DNA of bone marrow and intestinal mucosa cells during this period. Other rats were infused with TdR at 200 mg/kg/hr for 24 hours. Two-hour labeling with ^{32}P during continued TdR arrest revealed an 86% reduction in DNA synthetic activity of the bone marrow. A similar decline of 65% in activity was observed in the intestinal mucosa. In contrast, there was an increase in bone marrow DNA synthesis of up to two times that of control during periods following TdR release. These results are comparable to those observed with bone marrow DNA histograms during the release period. DNA synthesis in the intestinal mucosa is increased even further with release up to values seven times control values after completion of the TdR infusion.

Effect of TdR on the growth of myeloblastomas.—The study of cell kinetics during cell cycle traverse arrest and synchronous growth can also be applied to tumor cell populations. Rats bearing transplantable subcutaneous myeloblastomas (21) were monitored for the effect of TdR on tumor growth. Control rats were infused with saline. These subcutaneous myeloblastomas reached a 0.5-cm diameter at approximately 7 days following tumor

TABLE 2.—In vivo incorporation of ^{32}P into bone marrow and intestinal mucosa cell DNA as % of control during TdR arrest and after release*

Period	Bone marrow	Intestinal mucosa
During TdR arrest	14.4 ± 8.5	34.9 ± 7.57
Time after TdR release (hrs)		
2	95.9 ± 5.2	128.5 ± 36.6
4	120.5 ± 5.5	794.5 ± 83.7
6	192.3 ± 33.6	702.0 ± 189.4

*Rats (175–200 g) were infused with TdR at 200 mg/kg/hr for 24 hrs, then given 250 microcuries of ^{32}P by ip injection. The incorporation of ^{32}P into DNA during TdR arrest was measured by continuing the TdR infusion for an additional 2 hrs and then harvesting the bone marrow and intestinal mucosa. Similar incorporation studies during TdR release were performed by completing the TdR infusion at the time of ^{32}P injection and then killing animals at the appropriate time intervals to obtain tissue samples. The appropriate control animals were infused with saline. Values = mean ± SE of determinations obtained on 3 animals, expressed as % of control. The ^{32}P counts (mean ± SE)/10^6 cells obtained for the controls, 3 animals each at 2, 4, and 6 hrs after labeling, were: 2593 ± 674, 4957 ± 2269, and 6330 ± 280, respectively, for the bone marrow, and 204 ± 50, 275 ± 79, and 974 ± 245, respectively, for the intestinal mucosa.

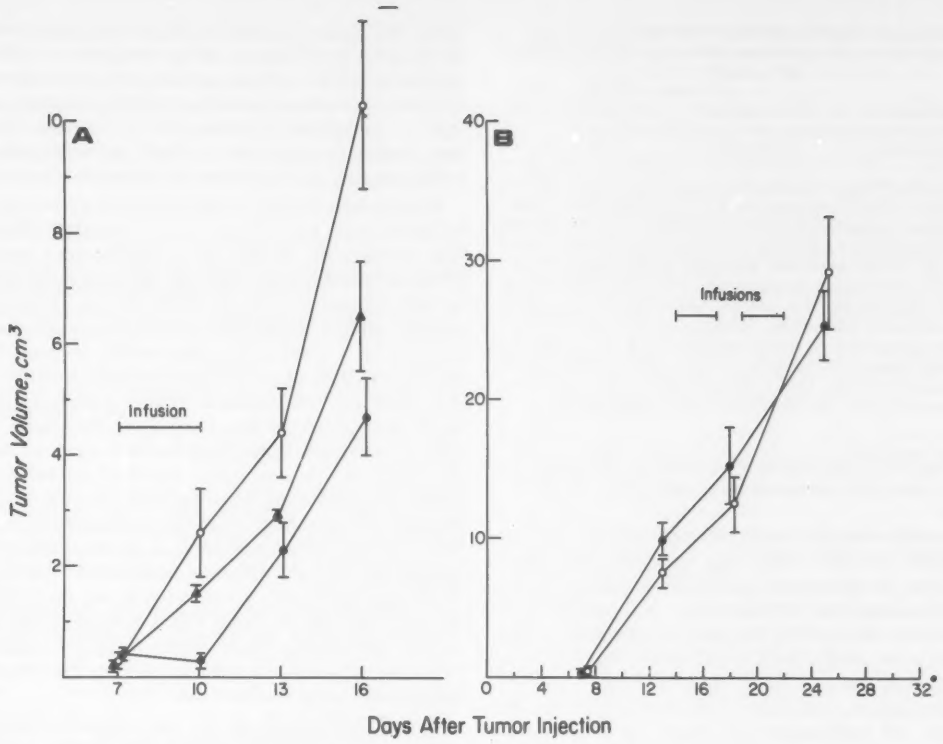

FIGURE 5.—Rats were inoculated sc at a single infrascapular site with a 1.0-ml suspension of 1×10^7 Wistar/Furth acute myelogenous leukemia cells (21). The sc myeloblastomas were measurable, with a 0.5-cm diameter at 7 days after tumor inoculation. Five rats were randomized to the different treatment groups. Values = mean tumor volume ± SE. (A) Infusions of saline (○), TdR at 300 mg/kg/hr (▲), or TdR at 600 mg/kg/hr (●) were begun on Day 7 after tumor inoculation and continued for 72 hrs. (B) Infusions of saline (○) or TdR at 600 mg/kg/hr (●) were begun on Day 14 for 72 hrs and again on Day 19 for 72 hrs.

injection. TdR infusions of 200 mg/kg/hr failed to detectably arrest the progression of tumors of this size. However, as shown in figure 5, inhibition of tumor growth was obtained during infusions at 300 mg/kg/hr, and there was an even more complete inhibition of myeloblast cell growth during infusions of 600 mg/kg/hr. At the higher dose rate, the 2-hour ^{32}P incorporation into myeloblast DNA (table 3) was 19% that of control values after 24 hours of infusion, confirming the arrest of tumor DNA synthesis. As the serum TdR levels were increased from 10^{-4} to 10^{-3} M, there were further declines in DNA synthetic activity of the bone marrow and intestinal mucosa, with values at the higher dose rate of approximately 7% and 23% of control, respectively. As noted in figure 5A, tumor growth was rapid following the infusions and reached the control values by 24 days after tumor injection. The sensitivity of the subcutaneous myeloblastomas to TdR was dependent, in part, on the tumor volume. The TdR infusion was begun at 600 mg/kg/hr on Day 14 after tumor injection, when tumor volume was ten times greater than that used in the previous studies (fig 5B). There was no measurable alteration in tumor growth despite the administration of multiple infusions at this dose because, under these conditions, tumor growth proceeded in a pattern similar to that of the control.

DISCUSSION

TdR was given by continuous infusion to determine whether the nucleoside can arrest cell cycle traverse of normal cell populations in vivo and whether synchronous growth would also occur in vivo after exposure of cells to TdR. Rats were infused with escalated doses, and the bone marrow

TABLE 3.—In vivo incorporation of ^{32}P into bone marrow, intestinal mucosa, and myeloblast DNA as % of control during TdR arrest*

Myeloblastoma	Bone marrow	Intestinal mucosa
19.1 ± 3.2	6.9 ± 3.2	22.6 ± 7.0

*Rats (175–200 g) were inoculated sc in the infrascapular region with a 1.0-ml suspension of 1×10^7 Wistar/Furth acute myelogenous leukemia cells and, 7 days later, were infused with TdR at 600 mg/kg/hr for 24 hrs. Control rats were infused with saline. All animals were then given 250 microcuries of ^{32}P ip, and, over a 2-hr period, incorporation of ^{32}P into DNA of bone marrow, intestinal mucosa, and myeloblastoma was measured during continued TdR arrest. Values = mean ± SE of determinations obtained on 3 animals, expressed as % of control. The ^{32}P counts (mean ± SE)/10^6 cells for the 3 controls infused with saline were 919 ± 152, 1864 ± 50, and 149 ± 14 for myeloblastoma, bone marrow, and intestinal mucosa, respectively.

was monitored for cytokinetic changes. Marrow cellularity was also measured on completion of the infusion.

Bone marrow cell counts declined with increasing TdR dose, and the effect was confirmed by hypocellularity on histologic specimens of the marrow. Statistical analysis revealed that the bone marrow depression was directly dependent, at a highly significant level, on duration of exposure after 24 hours and on dose rate. This finding is subject to two interpretations. Inhibition of DNA synthesis would prevent cell replication and might result in a decrease in cellularity, considering the turnover of the marrow. The other alternative is marrow cell death due to TdR cytotoxicity. Pathologic examination of a variety of other organs failed to reveal significant evidence of cytotoxic effects, except for edema of the intestinal lamina propria. Denuding of the intestinal epithelium was also noted at lethal TdR doses.

The myelosuppressive effects on the bone marrow were also monitored by following peripheral blood cell counts. Peripheral blood leukocytes were reduced as a result of maintaining serum TdR levels at 10^{-3} M for 72 hours. However, similar reductions in blood cell counts were not observed when TdR serum levels were maintained at 10^{-4} M for the same time period or at 10^{-3} M for shorter periods of exposure. This finding further emphasizes the direct dependence of TdR dose rate and duration on bone marrow depression. The recovery of the peripheral cytopenia was rapid, with a return to near baseline levels at 48 hours after completing the infusion, followed by a rebound on Day 6 to a level greater than twice the baseline leukocyte count.

The cytokinetic changes induced in the bone marrow were confirmed by flow cytometric and DNA incorporation studies. Serum TdR levels of 10^{-4} M were sufficient to inhibit DNA synthesis and to block cells in S phase. After removal of TdR, a proportion of the cell population proceeded through S phase with an increase in percent of S phase cells and a concomitant decrease in G_0-G_1 cells. There was further progression through the cell cycle and reappearance of a G_2 phase 6 hours after release.

Comparable results were obtained by in vivo labeling of bone marrow as well as intestinal mucosa. The monitoring of ^{32}P incorporation into cellular DNA revealed a transient cell cycle traverse arrest in both tissues at TdR levels of 10^{-4} M. During the release period, bone marrow DNA synthesis increased to a level twice that of the control. This increased DNA synthetic activity reflects enhanced cell cycle traverse and conceivably accounts for the rapid correction of the peripheral cytopenia. An even more marked increase in DNA synthesis was observed in the intestinal mucosa, as cells progressed in a partially synchronous manner through S phase. The uniformity of the cellular release and the degree of synchronization is determined by the serum half-life of TdR. The half-life of TdR in the rat is approximately 45 minutes at serum levels of 10^{-4} to 10^{-3} M. A more rapid release from TdR arrest, therefore, could conceivably be achieved through rescuing cell populations by the administration of deoxycytidine (12).

Serum TdR levels of 10^{-4} M, already shown to arrest cell cycle traverse of bone marrow and intestinal mucosa, failed to detectably alter the growth kinetics of myeloblastomas transplanted subcutaneously in similar rats. A partial inhibition of tumor growth resulted from infusions at 300 mg/kg/hr, while inhibition was nearly complete at higher doses, resulting in serum TdR levels of 10^{-3} M. This effect has also been shown to be dependent on maintaining a minimal tumor burden. The arrest of myeloblast DNA synthesis at the higher dose was confirmed by in vivo ^{32}P incorporation studies. Under these conditions, DNA synthesis in bone marrow and intestinal mucosa was also subjected to a similar degree of inhibition. Further, the recovery of myeloblast growth was rapid following the TdR infusions, thus indicating a transient arrest of cellular growth similar to that observed with the normal cell populations.

The continuous infusion of TdR in vivo provides several opportunities to develop new therapeutic approaches to cancer. TdR has been used widely to study the kinetics of in vitro cell proliferation, but similar studies have not been undertaken in vivo. The clinical effects of TdR have recently been reported in patients with a variety of hematologic malignancies and solid tumors (26–28). Differential

cytotoxicity with TdR alone would be dependent on a greater sensitivity of tumor cell populations to TdR-induced cell cycle traverse arrest. The differences in recovery of normal and tumor cells following traverse arrest may provide other opportunities for administering TdR in combination with cell cycle-specific agents such as cytosine arabinoside.

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Phase I Study of Pentamethylmelamine[1,2]

Ronald S. Goldberg, John P. Griffin, Joseph W. McSherry, and Irwin H. Krakoff[3,*]

SUMMARY

Pentamethylmelamine is a soluble monodemethylated derivative of hexamethylmelamine. Its activity in animal tumors is similar to that of hexamethylmelamine. When given iv at a dose of 1200 mg/m² to patients with advanced cancer, it produced severe nausea and vomiting, severe psychotropic effects, and EEG changes. When this dose was given for 10 consecutive days, there was marked but reversible myelosuppression. The severe CNS effects which occurred at doses sufficient to produce myelosuppression cast doubt on the potential utility of pentamethylmelamine.

[Cancer Treat Rep 64:1319–1322, 1980]

Clinical trials of hexamethylmelamine (HMM) demonstrated activity in small cell carcinoma of the lung, ovarian adenocarcinoma, lymphoma, and breast cancer (1,2). Because of its limited solubility, the drug was administered orally. With oral administration, gastrointestinal side effects were produced, and, most recently, marked variability in absorption was demonstrated (3). Attempts were made to develop a soluble form, using gentisic acid complexes, but these complexes were unstable at physiologic pH (4). In the search for active analogs of HMM with greater water solubility, pentamethylmelamine (PMM), a monodemethylated analog of HMM, was chosen as one of the most promising analogs because it is 24 times more soluble than HMM in water and shows similar activity against the P246 tumor [a human lung carcinoma grown in the nude mouse (5)], against Sarcoma 180 (6), and against a spectrum of other animal tumors.[4] It was more active in daily or intermittent treatment regimens than in a single or multiple dose on 1 day.

The mechanism of action of PMM is unknown. It was inactive in the *p*-nitrobenzylpyridine chemical test as an indication of alkylating ability (7). In tests as a folic acid antagonist, it had little activity in P388 leukemia.[5]

The preclinical toxicologic evaluation of PMM was performed in CDF mice, beagle dogs, rhesus monkeys, guinea pigs, and rabbits.[4] The clinical manifestations of toxic effects in dogs and monkeys occurred primarily during or following drug administration. These manifestations included anorexia, adipsia, diarrhea, emesis, tetanic seizures, hypothermia, weakness, disorientation, melena, and ataxia. CNS toxicity was rate-dependent: rapid injection could produce acute toxicity even at relatively low doses. Even at the acute lethal dose (4000 mg/m²) in the dog, giving the same dose over longer periods spared the animal. However, one half that dose rapidly injected proved to be lethal. Overall, drug administration resulted in toxic effects at the injection site and on the lymphatic, renal, male reproductive, and central nervous systems, with equivocal cardiovascular, respiratory, and gastrointestinal system toxicity. Clinical symptoms were present at most dose levels but could be avoided by spacing treatments. Hematologic and biochemical parameters were only transiently af-

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[4]Clinical brochure: pentamethylmelamine. Prepared by the National Cancer Institute, Bethesda, MD, June 1978.

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[5]Progress Report No. 10. Southern Research Institute, Birmingham, AL, Nov 1977.

fected, and PMM did not markedly affect rapidly proliferating tissues. The toxic dose low for the most sensitive species tested (beagle) was 125 mg/m² daily for 10 days.

This phase I study was undertaken to determine the characteristics of toxicity, the minimum dose which produced consistent, reproducible evidence of biologic effect, and the maximally tolerated dose in man, when given daily for a 10-day course.

MATERIALS AND METHODS

All patients selected for this study had advanced, histologically proven cancer for which conventional therapy had been exhausted or for which there was no conventional or investigational therapy with a reasonable expectation of benefit. Patients were > 18 years old and had a life expectancy of at least 12 weeks, a Karnofsky performance status of ≤ 30, and had had no chemotherapy or radiotherapy within the previous 30 days.

PMM was given iv. Each 500-mg vial was reconstituted with 9.5 ml of sterile water, yielding a solution containing 50 mg/ml. The daily dose was further dissolved in 250 ml of 5% dextrose and given by slow iv infusion over periods of 1, 2, and 6 hours. The daily dose was repeated for 10 days, and each course was repeated no more frequently than every 3 weeks. The starting dose was 40 mg/m² (⅓ the toxic dose low in the dog), and dose escalation followed a modified Fibonacci scheme. Three patients were given the drug at dose levels of 40, 60, 100, 150, 225, 350, and 500 mg/m²; four patients were given 750 mg/m²; and five patients were given 1200 mg/m².

Baseline data included history, physical examination, and review of the microscopic slides used to establish the diagnosis. Initial laboratory studies were cbc, bone marrow examination, urinalysis, SMA-12 and SMA-6, and other necessary studies based on tumor type and measurement of the extent of tumor. Followup studies included daily discussion of symptoms, evaluation of sensorium, physical examination, cbc, and urinalysis; weekly SMA-12; EEG, computerized tomographic (CT) scan, and lumbar puncture if neurologic changes were present; and appropriate roentgenograms, scans, etc, as necessary to evaluate changes in organ function which might be due to growth or regression of tumors or to drug toxicity.

RESULTS AND DISCUSSION

Of 17 patients enrolled in this study, 16 were evaluable; one patient did not complete a course because of nausea and vomiting due to a duodenal ulcer. Thirty-three of the 34 courses of PMM administered were evaluable (table 1). The most severe toxic effects were in the CNS. Nausea and vomiting was first noted at 350 mg/m² and increased in severity with increasing doses. The severity could be minimized slightly with 6-hour rather than 2-hour infusions and seemed to lessen in the latter days of therapy, but as the dose increased, the nausea and vomiting became severe and was not ameliorated by slowing the infusion. Compazine was ineffective in preventing nausea and vomiting.

At doses of ≥ 750 mg/m², additional CNS effects became evident. These included ataxia, claustrophobia, hyperexcitability, poor concentration, light-

TABLE 1.—PMM toxicity

Dose (mg/m²/day × 10)	No. of courses	No. of courses			
		Myelotoxicity		Nausea and vomiting	Neurotoxicity*
		Wbc	Platelet		
40	3	0	0	0	0
60	3	0	0	0	0
100	3	0	0	0	0
150	3	0	0	0	0
225	3	0	0	0	0
350	3	0	0	3	0
500	3	0	0	3	0
750	4	0	0	2	2
1000	1	0	0	1	1
1200	5	3†	2‡	5	4

*Signs of neurotoxicity: ataxia, weakness, light-headedness, poor concentration, "wild dreams," slurred speech, and somnolence.

†In these patients, wbc counts were 0.5 × 10^3/mm³, 1.8 × 10^3/mm³, and 3.2 × 10^3/mm³.

‡In these patients, platelet counts were 35 × 10^3/mm³ and 42 × 10^3/mm³.

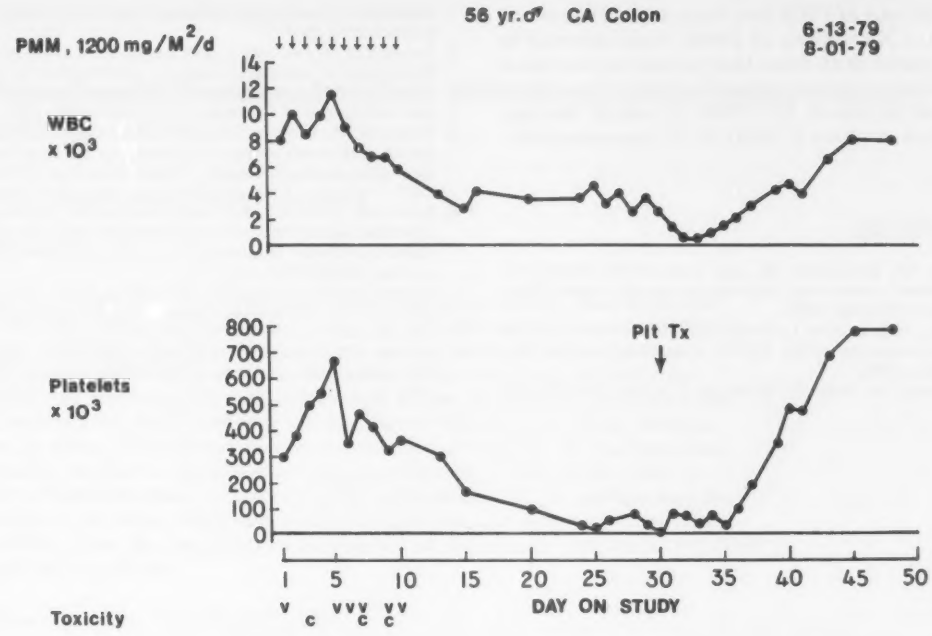

FIGURE 1.—Wbc and platelet counts in a patient who received 1200 mg/m² iv daily × 10 of PMM. In addition to myelotoxicity, severe nausea and vomiting and CNS effects (light-headedness, confusion, "wild dreams," ataxia) occurred.

headedness, "wild dreams," and slurred speech. Lumbar puncture and CT scans of the brain were unremarkable. However, EEG findings were positive. Single infusions for 2–6 hours of 1–1.2 g/m² of PMM elicited slowing of background rhythms by 1–2 cycles/second. In patients manifesting confusion and features suggestive of an organic brain syndrome, the EEG showed poorly reactive background rhythms in the 5–7-cycle/second range and prominent, intermittent frontal delta activity. The changes resembled those in toxic and metabolic states such as hepatic encephalopathy and hypercalcemia. Although several patients had metastases to the liver and abnormal liver function tests, the encephalopathy appeared to be directly related to PMM because the EEG changes in patients studied before and after infusion could be documented. There was gradual improvement in clinical and EEG findings in patients followed after a course of therapy. The mechanism of induction of the physiologic changes in the CNS is not clear because neither cholinergic nor anticholinergic agents give rise to these precise findings. The effects appeared to be quite long-lasting: in the more severely involved individuals, return toward normal occurred over a period of several weeks.

Myelotoxicity was not seen at low doses but was noted consistently at 1200 mg/m². Platelet and wbc counts were affected in parallel. They decreased within 5–7 days after completion of a 10-day course and remained depressed for 2–3 weeks (fig 1). There was no correlation of myelosuppression with abnormal liver functions or other abnormal biochemical parameters. The patients in this study had adenocarcinomas of the colon (ten), kidney (two), breast (two), ovary (one), and lung (two). There were no objective responses.

The reason for studying PMM in man was the expectation that its solubility might provide an iv preparation as effective clinically as HMM, without the erratic absorption which occurs when HMM is given orally. HMM must be given orally because of its limited solubility. The general clinical experience has been that HMM produces useful clinical effects only at doses which produce some myelosuppression (8). Animal studies suggested that the same thing

would be true of PMM (*see* footnote 4). The severe, limiting CNS toxicity of PMM which occurred in these studies at all doses that produce any evidence of myelosuppression makes it unlikely that a useful role will be found for PMM in cancer therapy. Therefore, a phase II study is not recommended.

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Indomethacin Treatment of Rats With Dimethylhydrazine-Induced Intestinal Tumors[1,2]

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SUMMARY

Intestinal tumors were induced in male Sprague-Dawley rats following the administration of five weekly doses of 1,2-dimethylhydrazine (DMH) by gavage. At intervals thereafter, groups of rats were given indomethacin (IND) in the drinking water. In three trials, the incidence of rats with tumors was significantly reduced by 40% below the control rats which did not receive IND and there was no significant difference in body weights. Also, in those IND-treated rats which developed tumors, the tumors were generally smaller in numbers and sizes compared to the control rats. A group of DMH-treated rats was treated with crude IND by gavage and the differences between them and untreated control rats were not significant. It is likely that the treatment was directed at the tumors and not at the DMH which induced them.

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Two model systems have been developed for experimental intestinal cancer: (*a*) autochthonous tumors induced in mice and rats by dimethylhydrazine (DMH) (1–4) and (*b*) transplanted colon tumors derived from the autochthonous tumors (5,6). The primary, or autochthonous, tumor system in rats resembles many aspects of the counterpart disease in man. This model system has been applied to two facets of research: (*a*) in vivo inactivation of DMH by carbon disulfide and related compounds (7) and (*b*) reversal of DMH-induced tumors. In tumor reversal, drugs and specific dietary components have been administered to experimental animals in an attempt to alter the pattern of tumor development: these included vitamin A (8), bran (9,10), butylated hydroxytoluene (11), and cytotoxic drugs (12) (M. Pollard, unpublished information, 1978). The reports indicated that the treatments produced little or no modification of the disease in tumor-bearing animals. Tumor induction by DMH was inhibited by feeding rats a chemically defined diet (13). Immunotherapeutic trials with BCG and *Corynebacterium parvum* in rats with DMH-induced tumors demonstrated that tumor growth could be enhanced by such treatments (14–16). In rats which were given DMH, the resulting tumor development and growth were promoted by administration of cholestyramine (1,17), high-fat diets (18), and sodium barbiturate (19).

We have used the DMH-induced model tumor system to examine the role of a prostaglandin inhibitor, indomethacin (IND), in the pathogenesis of disease. Earlier reports have indicated that intestinal and other tumor cells in man and in animals produced prostaglandins (20–24). There is evidence that prostaglandin inhibitors suppress the growth pattern of transplanted tumors (25–27). Treatment of tumor-bearing rats with IND has retarded the rate and extent of metastasis of prostate adenocarcinoma cells (28). We do not know if this effect was directed at the tumor cells or was mediated through host response to the drug. Contrary results, that prostaglandins inhibit tumor cell growth, have also been reported (29–31).

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The results of our tests indicate that, in rats, the number of DMH-induced intestinal tumors was reduced and that a significant number of treated rats that had received DMH were actually tumor-free. In the experimental protocol used, rats were given DMH for a 5-week period and, at intervals thereafter, groups of them were given continuous treatment with IND in the drinking water; the control rats consumed drug-free water. One group of rats was given crude IND by gavage at daily intervals.

METHODS

Earlier tests demonstrated that male Lobund Sprague-Dawley (S-D) rats were more susceptible to DMH-induced intestinal carcinogenesis than female S-D rats and male Buffalo and Wistar rats (32). Intestinal tumors developed in all S-D rats following administration of ten or five weekly doses of DMH (30 mg/kg of body weight/week) by gavage (table 1) (19). The number of tumors induced per rat was higher in response to ten doses than to five doses of DMH; however, the spectrum of tumor locations, sizes, and types induced in the rats was the same.

The S-D rats were produced in a closed, isolated colony which was random-bred through 36 generations. They accept reciprocal skin grafts. Aged S-D rats have been examined for spontaneous tumors during the past 10 years, and no intestinal tumor has been observed among them. They were free of pathogenic microflora. They were fed, ad libitum, Tek-Lad (L-485) diet and tap water. They were housed in plastic cages on ground corn cobs, in an air-conditioned room with 12-hour intervals of light and darkness.

During the period of exposure to DMH, the rats were maintained in plastic isolator enclosures from which the effluent air was filtered and discharged to the environment from the roof of the building.

Experimental Protocol

Weanling conventional male S-D rats were given five doses of DMH[4] (30 mg/kg/week) by gavage. The DMH was prepared fresh in sterile physiologic saline prior to administration. At intervals of 3, 12, and 35 days after the fifth dose of DMH, groups of rats were given, ad libitum, purified IND[5] in the drinking water (20 mg/liter). The IND was dissolved in a small amount of absolute ethanol which was then added to the tap water (27). Fresh drug-treated water was provided at 3-day intervals. Control rats were given drug-free tap water which was not chlorinated or acidified.

One group of S-D rats received five doses of DMH according to the protocol noted above. At 7 days after the fifth dose of DMH, a group was given IND (0.25 mg/kg) by gavage once each day. The drug was taken from commercial capsules of IND (Indocin)[5] suspended in 1% cornstarch. Control rats were given 1% cornstarch.

Twenty weeks after the start of each experiment, each rat was weighed and killed by deep ether anesthesia and exsanguination from the exposed heart. The entire intestinal tract was excised and opened from the anus to the stomach. The intestinal contents were washed away, and the intestines were examined at 3× magnification for number and location of lesions. This information and weights of livers were recorded. The tissues were fixed in Bouin's solution for 18 hours and transferred to 70% ethanol. The fixed intestines were re-examined, the location and number of lesions were noted, and the lesions were processed for histologic examination.

The results with individual rats per group were analyzed statistically by Mann-Whitney significance tests.

Two groups of weanling S-D rats were fed, ad libitum, drinking water with IND (20 and 40 mg/liter) which was changed at intervals of 3 days. The rats were observed for 6 months. Sick rats were killed and examined at autopsy, and rats that survived the observation period were killed for examination at autopsy.

RESULTS

Effect of DMH

The DMH-treated rats survived the 20 weeks of the experimental protocol. Occasionally, toward the end of the observation period, DMH-treated rats developed diarrhea, and some of them had blood-stained feces. Those that had no further treatment developed neoplasms in the colon and duodenum, including small and large polyps, sessile tumors, and invasive mucus-producing tumors. Some of the tumors were 0.5–1 cm in diameter and were attached to the intestinal wall by a long slender stalk, and some extended into the serosal cover of the intestine. The range of intestinal tumors was similar to that described by Ward (33). There was no gross evidence of metastatic spread of tumor cells; however, clumps of tumor cells were detected micro-

[4]DMH (1,2-dimethylhydrazine hydrochloride) was supplied by Aldrich Chemical Co, Milwaukee, WI.

[5]Pure IND (99.7% pure—Lot No. L-590,226-00A125); and Indocin in capsules (25 mg/capsule, plus inert filler) was supplied by Merck Sharp & Dohme Research Laboratory, Rahway, NJ.

TABLE 1.—Dose response to DMH*

No. of DMH doses	No. of rats with tumors/ No. of rats inoculated	Total No. of tumors	Average No. of tumors in—	
			Tumor-bearing rats (range)	Inoculated rats
1	12/20	17	1.4 (1–2)	0.85
5	45/45	192	4.4 (1–10)	4.40
10	23/23	323	14.0 (4–27)	14.00

*Weanling male Lobund S-D rats were given DMH weekly by gavage (30 mg/kg/wk). At Week 20 after the first dose of DMH, each rat was killed and examined for intestinal tumors.

scopically in the adjoining lymph nodes in 5% of the rats. An occasional tumor was observed in areas of lymphocyte aggregations (Peyer's patches) in the wall of the proximal one third of the colon. None of the rats developed lesions in other organs.

Effect of IND

The DMH-treated rats which had been treated with IND appeared healthy. The weights of bodies and livers were comparable to counterpart weights in the DMH-treated rats which were not treated with IND. The number of tumor-bearing rats was reduced by at least 40% ($P < 0.0016$), and the number of tumors per IND-treated rat was lower than in the control rats ($P < 0.002$, 0.0065, and 0.007) (table 2). In the group of rats treated daily with capsule-derived IND by gavage, the number of tumor-bearing rats was reduced by 40%; however, the difference in the number of tumors in the two groups was not significant ($P < 0.0821$).

Most of the tumors which developed in the IND-treated rats were smaller than those in the untreated controls, and they showed less activity as judged by numbers of mitotic figures. Exceptions to this were observed. Occasionally, an IND-treated rat had a very large tumor in the form of a diverticulum filled with amorphous cellular debris, polymorphonuclear leukocytes, and small discrete islands of intact neoplastic cells. Occasionally, an IND-treated rat had a large actively growing tumor similar to that observed more often among the rats which had not received IND.

A unique type of lesion was observed in the small

TABLE 2.—Effects of IND on development of DMH-induced intestinal tumors

Experiment No.	No. of rats	Interval between DMH and IND (days)	IND	Average weight (g)		No. of rats with tumors	No. of tumors in—		Average No. of tumors—	
				Body	Liver (% body weight)		Colon	Duodenum	Per rat	Per tumor-bearing rat
					*Protocol 1**					
1	10	3	+	402	11.2 (2.7)	6	7	1	0.9	1.3
	10		–	397	10.7 (2.6)	10	38	15	5.3	5.3
				0.8501†					0.002†	
2	10	12	+	390	12.3 (3.1)	5	7	2	0.9	1.8
	10		–	402	13.0 (3.2)	10	21	5	2.6	2.6
				0.4274†					0.0065†	
3	10	35	+	436	14.4 (3.3)	4	5	0	0.5	1.2
	9		–	415	14.2 (3.4)	9	11	9	2.2	2.2
				0.9342†		0.0016†			0.007†	
					Protocol 2‡					
	10	7	+	391	12.3 (3.1)	6	9	5	1.4	2.3
	10		–	396	12.6 (3.1)	10	22	5	2.7	2.7
				0.7624†		0.1405†			0.0821†	

*Weanling male S-D rats were given DMH by gavage (30 mg/kg/wk × 5). Then, at intervals, a group of the rats were given, ad libitum, water with IND (20 mg/liter); control rats were given drug-free water. The IND was changed at 3-day intervals. All rats were killed and examined at Week 20.

†Test is significant at level indicated.

‡At 7 days after the 5th dose of DMH, 10 rats were given IND by gavage (0.25 mg/kg). The drug was removed from commercial capsules of Indocin and suspended in 1% cornstarch. Control rats were fed 1% cornstarch. All rats were killed and examined at Week 20.

intestines of all IND-treated rats. These lesions appeared as distinct "knobs" protruding on the serosal surfaces of the intestine, ranging from 0.2 to 0.5 cm in diameter and from one to seven in number per small intestine. Occasionally, the knobs were hollow, but most were solid. The mucosal surface over the knob area was depressed, and gross examination showed no evidence of hemorrhage or surface erosion in the affected areas. Microscopic examinations showed that the sectioned knobs contained connective tissue, capillaries, and polymorphonuclear leukocytes. Some areas leading into the knob had no epithelial mucous membrane barrier. The intestinal muscularis layer around the knob was not clearly defined. Active and intact Peyer's patches were observed in the intestines of the IND-treated rats, but the number was reduced, and some were almost replaced by knobs of granulomatous tissue, which extended beyond fragments of intestinal muscularis in sections prepared with Masson trichrome stain.

The normal rats that consumed IND (20 mg/liter) in drinking water survived for 6 months with no evidence of clinical illness; however, autopsy findings showed that they had developed small knobs in the small intestines. Groups of rats were examined at Day 25 after the start of IND treatment, and none had visible lesions in the intestine. They consumed an average of 0.502 mg/rat/day. On Day 9, the condition of the ten rats that consumed water with 40 mg of IND/liter worsened, and all of them were dead by Day 35. They had developed peritonitis, with considerable purulent material in the abdominal cavities and extensive intestinal adhesions. They had consumed an average of 0.670 mg/rat/day.

DISCUSSION

The data in this report involved primary (autochthonous) tumors which were induced in the intestines of rats by orally administered DMH. The tumors were multiple sizes and types, and a small percentage (about 5%) had metastasized to adjoining lymph nodes. The five-dose schedule of DMH, which induced tumors in all rats (table 1), was used to avoid an overwhelming burden of tumors on the subsequent therapeutic regimen.

The dose schedule of IND (20 mg/liter of drinking water) was determined arbitrarily. It is estimated that an adult rat consumes 30 ml of water/day; thus each rat would consume 0.3 mg of IND/day/100 g of body weight, which is a larger dose than that given daily by gavage in 1% cornstarch (0.025 mg/100 g). Thus, the significant benefit of giving IND in drinking water over giving it by gavage may be either dose-related or related to the purity of the two drug forms used: 99.7% purity versus that derived from capsules. The drug-treated rats were not affected adversely by either IND treatment schedule, as evidenced by weights of bodies and livers and by the absence of acute ulcerating lesions in the intestines and stomach.

The design of this experiment provided for exposure of the test animals to DMH during a predetermined period. After a designated interval, they were given IND. It is likely that with such a time schedule, the IND effect was directed at the tumors, not at the agent responsible for them. Since five doses of DMH induced intestinal tumors in all of the S-D rats, the 40% reduction of tumor-bearing rats through treatments with IND is significant, and this was confirmed statistically in three trials (table 2). Also, the IND-treated tumor-bearing rats had fewer tumors than the control rats (table 2).

The knobs on the serosal surfaces of small intestines in IND-treated rats are of uncertain nature. These lesions may actually represent (*a*) abortive tumors, or (*b*) reparative tissue responses to ulcerogenic changes, or (*c*) intestinal diverticula. The lesions were observed predominantly in IND-treated rats, and many of them were located in areas of Peyer's patches. Rats which were fed double the dose of IND in drinking water (40 mg/liter) for 3 weeks developed intestinal perforations and peritonitis with extensive adhesions. Rats given 20 mg of IND/liter of drinking water did not develop these acute lesions in the intestinal tract.

A number of issues need clarification. (*a*) Is the IND treatment only suppressive to tumors, and will they re-appear after termination of the drug treatment? (*b*) Will IND treatment prevent DMH carcinogenesis? (*c*) Do the DMH-induced intestinal tumors produce prostaglandins? (*d*) Do the tumors observed in IND-treated rats produce prostaglandins? (*e*) Will other nonsteroid anti-inflammatory agents show therapeutic benefits? (*f*) Will IND interfere with the development of intestinal tumors induced by other carcinogenic agents?

Prostaglandins have been described as immunosuppressive agents (34,35). If development and growth of intestinal tumors are regulated by immune mechanisms, then IND treatments may function by protecting the immune (antitumor) status of the host.

The model tumor system used here provides the means for further studies on pathogenesis of tumor formation and on the identification of therapeutic and promoting agents. As reported here, it has identified a possible therapeutic agent which warrants further attention.

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Effect of Microbial Fractions and Vehicle on Survival of Mice Bearing Lewis Lung Carcinoma[1,2]

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SUMMARY

Mice bearing pulmonary metastases of the Lewis lung carcinoma were treated iv with a nonviable microbial vaccine following amputation of the primary inoculation site. The vaccine consisted of BCG cell wall skeleton, trehalose dimycolate, and endotoxin attached to mineral oil microdroplets. Single and repeated doses ranging from 15 to 675 μg were tested. Survival was modestly but consistently prolonged by vaccination. A portion of the activity appeared to be due to the Tween saline-oil vehicle. A single low dose (15 μg) was as efficacious as higher or repeated doses.

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The modest efficacy of viable BCG in the treatment of experimental and human malignancy has stimulated investigation of BCG extracts in an effort to increase therapeutic index. One such extract is BCG cell wall skeleton (CWS), the product of enzyme and organic solvent treatment of whole BCG. CWS, combined with mineral oil droplets in an oil-in-water emulsion, is capable of shrinking both line-10 guinea pig hepatoma (1) and human melanoma (2) when it is injected into the tumor nodules. In some circumstances the addition of trehalose dimycolate (TDM) to the CWS-oil preparation increases tumor regression (3). The addition of mutant bacterial endotoxic glycolipids to this preparation may further increase the activity of the vaccine when it is injected into the dermal nodules of the line-10 hepatoma (4).

Since clinical malignancy is basically a problem of visceral metastases, we tested the CWS-TDM-endotoxin preparation in oil for its activity against visceral tumor. The present study was designed to evaluate the effect of iv CWS-TDM-endotoxin vaccine on the survival of mice bearing Lewis lung carcinoma (LLC). LLC has characteristics which make it a potentially good model for immunotherapy. It is a spontaneous tumor passed in a syngeneic host (5). Injected sc or im, it metastasizes to the lung, providing a model for visceral disease. In vivo, the tumor is poorly immunogeneic, as measured by the failure to demonstrate resistance to a second challenge in mice cured by surgery and chemotherapy (6).

Mycobacterial vaccines are active in LLC. Serrou et al (7) have shown that 1 mg of Pasteur BCG administered iv, in conjunction with radiation to the local tumor, resulted in a significantly decreased number of lung metastases but did not affect survival. Treves et al (8) showed that small doses (0.2 mg) of ip methanol extracted residue (MER) were not more effective than large doses (0.4 mg) in reducing the incidence of fatal pulmonary metastases in LLC. To be effective, the MER had to be given just before or just after amputation of the inoculated limb. Mathé et al (9) achieved an increased lifespan of 20% with MER administered 3 days before inoculation of mice with LLC. *Corynebacterium parvum* administered iv or ip was also effective in LLC (10). In this model, the tumor-bearing site on the flank was excised 10 days following inoculation.

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Vaccination 3–4 days prior to tumor excision resulted in a significant reduction in the number of pulmonary metastases measured on Day 21 or 28. *C. parvum* administered on the day of excision did not significantly reduce the number of metastases.

In the present study, we have used LLC as a model for treating pulmonary metastases following amputation of the primary inoculation site on the limb. The effect of iv CWS-TDM-glycolipid vaccine on survival was measured. The sequence of primary tumor, amputation, and immunotherapy was chosen because it simulates the conventional clinical experience and subjects the mouse to a primary tumor burden prior to "adjuvant" treatment of pulmonary micrometastases.

MATERIALS AND METHODS

The vaccine components were supplied by Edgar Ribi and Hamilton Biochemical Research Laboratory, Hamilton, MT, and were combined according to the method of Myer et al (3). The CWS, TDM, and endotoxin were dissolved together in chloroform ethanol in a 1:1:1 ratio. The solvent was then evaporated, and the dessicated powder was ground with a Teflon pestle with sterile Drakeol 6-VR light mineral oil. The oil and the water emulsion was then formed by the addition of Tween saline (Tween 0.2%, GIBCO) during the grinding procedure. Depending on the experiment, the concentration of vaccine varied from 75 to 3375 μg/ml. The amount of oil was kept constant at 0.75% by volume.

Mice were 8-week-old C57BL/6 males supplied by Jackson Laboratories, Bar Harbor, ME. Tumor was passaged by sterile dissection from the leg of a killed mouse, mincing by passage through wire mesh, and sc injection of 0.1 ml of a 10% suspension into the right upper hind limb. Palpable tumor appeared at the site of the injection 5–7 days later. This was designated Day 0 of the experiments. On Day 5 (10–12 days after inoculation), the tumor-bearing limb was amputated by hot cautery (11). Ip sodium pentobarbital (100 mg/kg) was used as general anesthesia. By this time, all animals had developed pulmonary micrometastases, and amputation was not curative (12). Following surgery, animals were randomized to experimental groups of ten to 15 animals each. Mice were injected with Tween saline-oil vehicle (vehicle control) or various doses of vaccine or were left uninjected (surgical control).

All volumes of vehicle and vaccine injections were given in 0.2-ml doses on Days 7, 11, or 14 following appearance of palpable tumor (*see* Results). Iv injection was performed with a 25-gauge needle and tuberculin syringe after warming the tail. Animals that died were autopsied. Animals dying of tumor had grossly visible pulmonary metastases, usually associated with hemorrhage. A two-tailed Wilcoxon-Gehan test (13) was used to analyze survival curves. Increased lifespan (ILS) was calculated as percent change in the median survival in the vaccine-treated groups over that of the vehicle control or surgical control groups.

Three series of experiments were performed to test the effect of vaccine and Tween saline-oil vehicle administration on survival. The effects of dose, timing, and repetition of dose on survival of tumor-bearing animals were tested.

RESULTS

Figures 1a and 1b show survival curves, from two experiments, for surgical control, vehicle control, and vaccine-treated animals injected with 15 μg of vaccine on Day 7 following appearance of tumor (2 days following amputation of the tumor-bearing limb). In both experiments, the survival of the animals treated with vaccine is significantly superior to that of the amputation control groups (P = 0.002). In figure 1a, the survival of the vaccine-treated animals is also superior to that of the vehicle controls (P = 0.01). However, the survival of vaccine-injected animals is not significantly superior to that of the vehicle controls because the vehicle injection also prolonged survival (fig 1b).

The effects of vaccine and vehicle on survival are shown in more detail in table 1. Various doses and schedules were tested in three experiments. The ILS of vaccine-treated mice over the median survival of both surgical and vehicle controls is shown for each treatment group. The median survival times of surgical control groups for the three experiments were 23, 24, and 19 days, respectively. In eight of the nine animal groups in the three different experiments, the survival of vaccine-treated animals is significantly superior to that of surgical control groups. In three treatment groups (a, d, and e) this difference is nullified if the group receiving the vehicle is used as the control. In experiment 2, the efficacy of two different vaccine doses was examined and the effects of single versus repeated administration were tested. The effects of the larger dose (225 μg) were not superior to those of the smaller dose (15 μg). The ILS for the 15-μg dose was actually greater. Moreover, repeated doses added no ILS, compared to the effects of a single dose and, if anything, were less effective.

In experiment 3, the effects of varying vaccine doses were examined and the effects of varying the

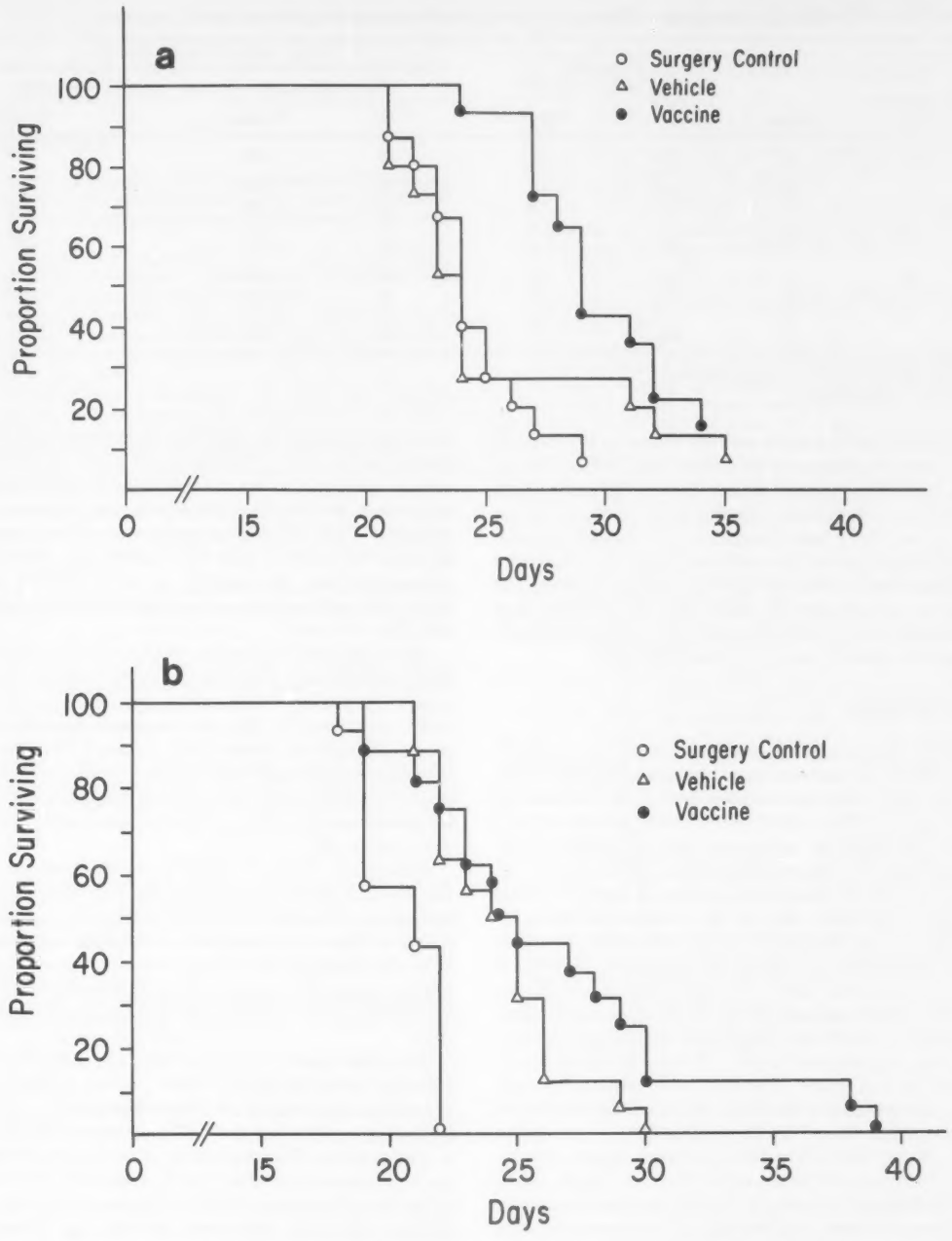

FIGURE 1.—Survival of C57BL/6 mice following tumor inoculation and amputation of the inoculated limb; uninjected and vehicle- and vaccine-injected (15 μg) groups are compared. *a*. Vaccine vs surgical control ($P = 0.002$); vaccine vs vehicle control ($P = 0.01$); and vehicle control vs surgical control (NS). *b*. Vaccine vs surgical control ($P = 0.002$); vaccine vs vehicle control (NS); and vehicle vs surgical control ($P = 0.002$).

TABLE 1.—Comparison of survival of vaccine-treated mice with surgical and vehicle controls

Experiment No.	Group	Treatment		ILS of vaccine-treated mice vs—			
		Dose (μg)	Day*	Surgical control		Vehicle control	
				%	*P* value	%	*P* value
1	a	15	7	29	0.002	0	NS†
2	b	15	7	21	0.002	21	0.01
	c	225	7	12	0.028	12	0.045
	d	15	7, 14	19	0.01	12	NS
	e	225	7, 14	21	0.04	14	NS
3	f	66	7	26	0.006	21	0.002
	g	675	7	22	0.015	17	0.002
	h	66	11	17	0.03	8	0.04
	i	675	11	13	NS	4	NS

*No. of days after tumor appearance.
†NS = not significant.

timing of vaccine were tested. Doses of 66 and 675 μg were administered on either Day 7 or 11 following the appearance of tumor (2 or 6 days after amputation of the tumor-bearing limb). Animals given 66 μg on Day 7 had the greatest ILS, although the results for both high and low doses were superior to those for controls on this day. However, delaying vaccine administration until Day 11 resulted in a diminished ILS and abrogated the survival advantage for animals receiving the high dose.

DISCUSSION

This therapeutic system was designed to evaluate the effects of vaccination on survival of mice bearing pulmonary micrometastases following removal of the primary tumor burden. The data indicate that the CWS-TDM-glycolipid vaccine has a statistically significant, but relatively modest, effect in prolonging survival in this tumor system. A portion of this effect is probably due to the vehicle. No clear increase in survival resulted by increasing the dose of vaccine above 15 μg or by repeating the dose. It is possible that there is a threshold above which dose is irrelevant except for its toxicity and timing. Treves et al (8) also found that timing was critical for the suppression of the LLC by ip MER. Iv *C. parvum* was more effective in decreasing pulmonary metastases in the LLC when administered 6–8 days (rather than 8–10 days) after tumor inoculation (10). Scott (14) found that repeated doses of iv *C. parvum* were no more effective than single doses in inhibiting the growth of a mouse mastocytoma.

The particular combination of components used in the present study was chosen because of data indicating that effects of this combination may be superior to those of any of its components in the line-10 hepatoma system (15). Whether all of these components are necessary for the therapeutic effect shown in the LLC remains to be seen. In another report, in which these components were tested as adjuvants for specific immunotherapy, the combination of CWS, TDM, and endotoxin did not appear superior to that of the CW alone (16). There is agreement that for activity in intratumoral systems, these extracts must be attached to oil droplets (17,18).

The mechanism of action of these vaccines is not fully understood. The efficacy of BCG given intratumorally is associated with a delayed hypersensitivity reaction (19). This is abrogated by cortisone or antithymocyte serum (20). In the LLC, the inhibition of pulmonary metastates by *C. parvum* is positively correlated with carbon clearance and can be abrogated by silica, cortisone, and antilymphocyte serum (21).

Endotoxin is also a known activator of macrophages (22), and it is possible that the various components in this vaccine simply represent the collection of additive or synergistic macrophage activators. It is also possible that tumor necrosis factor is generated since BCG and endotoxin administered sequentially are known to generate this factor in mice (23).

The prolongation of survival seen with vehicle injection could be due to Tween, which is known to potentiate the effects of chemotherapeutic agents, probably by altering the cell membrane (24,25). It is also possible that the stress of the injection itself may influence survival in LLC-bearing mice. Stressed mice have depressed, delayed hypersensitivity reactions (26) and depressed macrophage function (27).

It remains to be seen whether this modest prolongation of survival can be significantly improved by manipulating the vaccine components or sched-

ule of administration. It will also be important to determine if all the components are necessary for this effect and if simpler components such as muramyl dipeptide (28) can be substituted.

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Phase I Trial of Pentamethylmelamine in Patients With Previously Treated Malignancies[1]

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SUMMARY

Pentamethylmelamine (PMM), a demethylated soluble analog of hexamethylmelamine, was given to 35 patients with solid tumors in a phase I clinical trial. Thirty patients were given single doses ranging from 80 to 2000 mg/m² in a 2-hour infusion every 3 weeks. Once a maximum tolerated dose was defined for this schedule, an additional five new patients plus four patients who had already received PMM were treated on a multiple-dose schedule of PMM given three times a week every Monday, Wednesday, and Friday (M-W-F) for 4 weeks. Dose-limiting toxic effects for the single-dose schedule were in the central nervous system and gastrointestinal tract, manifested by nausea (60%), vomiting (49%), somnolence (37%), depression (6%), and headache (6%). Other toxic effects observed on this schedule included anorexia (34%), diarrhea (7%), and diaphoresis (21%). The toxic effects were first observed in mild form at 400 mg/m²/dose and became progressively more severe and prolonged with each dose escalation; they were considered intolerable at the 2000-mg/m² dose level in all patients treated. The nine patients receiving the multiple-dose schedule were given PMM at a dose of 1000 mg/m² three times a week (M-W-F). This level produced dose-limiting nausea and vomiting in all patients so that no patient completed > 3 weeks of treatment on this schedule. One patient developed PMM-related visual hallucinations. PMM produced no hematologic, hepatic, renal, allergic, or acute side effects; no alopecia was observed. Minor tumor regressions of 1 month's duration were seen in two patients, one with pleural mesothelioma and one with a parotid gland tumor. The recommended doses for solid tumor phase II studies are 1500 mg/m² given as a 2-hour infusion every 3 weeks and 1000 mg/m² given three times a week (M-W-F), repeated at 3-week intervals.

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Pentamethylmelamine (PMM) is a monodemethylated derivative of hexamethylmelamine (HMM), and was developed for clinical trials because of its greater solubility and consequent suitability for parenteral administration. Direct comparisons of PMM and HMM against a wide variety of rodent tumors indicate that their antitumor activity is quantitatively the same (1,2). HMM has been found to be effective against some human tumors (especially lung and ovarian) (3–5). However, the clinical use of HMM has been limited because of its insolubility in physiologically compatible vehicles and severe nausea and vomiting among patients (6). PMM was chosen for clinical study because of its parenteral dosage form and because of

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its solubility, stability, and effect against the human bronchial tumor (xenograft) (2).

Because the clinical spectrum and toxicity of HMM resemble those of alkylating agents, it was subjected to the *p*-nitrobenzylpyridine chemical test as an indication of its alkylating activity. Both HMM and its demethylated analogs are inactive (7). In addition, PMM has little effect on the uptake of tritiated thymidine, uridine, and leucine into P388 leukemia cells and therefore does not seem to act as a folic acid antagonist.[3] The mechanism of action of PMM and the parent compound HMM is therefore unknown.

Animal toxicity trials of PMM were conducted in beagle dogs and rhesus monkeys.[4] In dogs and monkeys, the clinical manifestations of drug toxicity occurred mainly during and immediately after PMM administration. In dogs, the toxic effects included mucoid stool, melena, polydipsia, fecal and urinary incontinence, ataxia, confusion, lethargy, paresis, convulsions, fever, and apnea. In monkeys, the toxic effects included hematochezia, anuria, and acoprosis. Both species exhibited anorexia, diarrhea, vomiting, weight loss, seizures, hyperexcitability, sedation, dyspnea, and injection site disturbances. The symptoms relating to nervous system stimulation were rate-dependent; ie, rapid injection produced stimulation even at low dose levels, but the same dose given over 1–2 hours had no immediate central nervous system (CNS) toxicity. Hematologic or clinical chemistry alterations were mild to moderate at sublethal dose levels.

The toxic dose low for dogs, the most sensitive species, receiving a single-dose schedule is 250 mg/m². One third of the toxic dose low (80 mg/m²) in dogs was used as the starting dose for this clinical phase I study.

PATIENT SELECTION AND EVALUATION

Thirty-five patients (22 males and 13 females), with a median age of 58 years (range, 33–74), received one or more doses of PMM. All patients had solid tumors (table 1). The median Karnofsky performance status of all patients entered in the study was 70% (range, 40%–100%). Criteria for entry in the study required histologic proof of malignant disease, lack of conventional therapy, no chemotherapy or radiotherapy for 4 weeks prior to entry in the study (8 weeks in the case of nitrosoureas), and a minimum life expectancy of 6 weeks. Informed consent was obtained in writing from each patient. Patients were expected to have acceptable blood cell counts (wbcs > 4000 and platelets > 100,000/mm³) and normal hepatic and renal functions. Measurable disease was not required. Twenty-six patients (83%) had received extensive prior chemotherapy, including nine (29%) who had received chemotherapy plus radiotherapy and two additional patients who had received radiotherapy only; three patients (10%) had not received either chemotherapy or radiotherapy.

Table 1.—Tumor types in patients treated with PMM

Disease	No. of patients	
	Total	Evaluable for response*
Colon	9	1
Melanoma	5	
Lung	9	
Adenocarcinoma	2	1
Squamous cell carcinoma	3	1
Undifferentiated cell carcinoma	1	
Small cell carcinoma	3	
Soft tissue sarcoma	2	1
Breast	1	
Renal	1	
Ovarian	1	
Parotid	1	1
Pancreatic	1	
Bladder	1	
Mesothelioma	1	
Unknown primary, adenocarcinoma	1	1
Laryngeal, squamous	1	1
Fibrohistiocytoma	1	1

*Criteria for response evaluation included measurable disease and ≥ 2 doses of PMM at 1500 mg/m² × 1 every 3 wks or ≥ 3 doses of PMM at 1000 mg/m² 3 times/wk with followup information for 4–6 wks.

Baseline studies obtained before initiation of treatment included: cbc, differential count, platelet count, urinalysis, serum electrolytes, glucose, BUN, calcium, phosphorus, uric acid, creatinine, bilirubin, albumin, total protein, alkaline phosphatase, SGOT, LDH, prothrombin time, partial thromboplastin time, and fibrinogen. During therapy the cbc, differential count, and platelet count were repeated twice weekly, and the coagulation and biochemical tests were repeated weekly.

DRUG AND SCHEDULE OF ADMINISTRATION

PMM was supplied by the Investigational Drug Branch, Division of Cancer Treatment, National

[3]Progress Report No. 10. Southern Research Institute, Birmingham, AL, Nov 1977.

[4]Clinical brochure: pentamethylmelamine (NSC-118742). Prepared by the National Cancer Institute, Bethesda, MD, 1978.

Cancer Institute (Bethesda, MD), in 20-ml vials as a lyophilized powder containing 500 mg of PMM and 300 mg of mannitol plus sodium hydroxide. Each 500-mg vial, when reconstituted with 9.5 ml of sterile water for injection (USP), contained 50 mg/ml of PMM and 30 mg/ml of mannitol and sodium hydroxide, for an adjusted pH of 4.5–5.5. The reconstituted solution was to be stable for at least 7 days at room temperature (22°C–25°C) or refrigeration temperature (2°C–8°C). Further dilution in 5% dextrose in sodium chloride injection, USP, did not alter stability. Since the supplied solution contained no antibacterial preservatives, the PMM was given within 4 hours after preparation. In this study, the drug was administered iv over 2 hours.

Patients usually received PMM as outpatients. Pulse, temperature, and blood pressure were recorded prior to administration and at 15–30-minute intervals thereafter as indicated. One course of treatment consisted of a single dose of PMM given every 3 weeks (table 2). At least three patients received each nontoxic dose level and were observed for 2 weeks before receiving a higher dose. Four to five patients were admitted at any dose level that showed reversible toxicity. One patient, who had received more than three doses of PMM at a single dose level without undue toxic effects and who had a minor tumor response, was escalated to the next available dose level; this patient then received two more courses at the new dose level.

After a maximum tolerated dose (MTD) was defined for the single-dose (every 3 weeks) schedule, an attempt was made to increase the total amount of drug given by using a multiple-dose (Monday, Wednesday, and Friday [M-W-F]) schedule given over 4 weeks. Five patients who were not previously treated with PMM and four patients who had already received one or more doses of PMM at ≥ 1500 mg/m² and who had tumor stabilization and/or subjective improvement were entered in this protocol.

RESULTS

Toxicity

Single-Dose Schedule (Every 3 Weeks)

Thirty patients received 56 courses of PMM (range, one to five) as a single dose every 3 weeks. All patients were followed for 4–6 weeks and are evaluable for toxic effects. Nonhematologic side effects are shown in table 3. Dose-related and dose-limiting toxicity was confined to the CNS. It was manifested as nausea and vomiting, somnolence, depression, headache, and confusion. Nausea and vomiting occurred in 17 patients (60%). The vomiting, which was first observed at the 400-mg/m² dose level, usually began at the end of PMM administration and persisted for several hours. However, as the dose of PMM was escalated, the onset of vomiting progressively advanced so that at 2000 mg/m² it began after 1 hour of the 2-hour infusion and persisted for 24–30 hours irrespective of antiemetic medications. Other CNS toxic effects were not observed at doses < 1000 mg/m² except for one patient with severe liver impairment (bilirubin 1.7 mg/100 ml; SGOT > 500 mg/100 ml; and LDH > 1000 mg/100 ml) who experienced a syndrome of weakness and somnolence but not confusion for 3 days after receiving PMM at 560 mg/m². She died after one course of progressive melanoma. Four of

TABLE 2.—Dose increments of PMM and No. of patients treated at each level

	Dose (mg/m²/day)	No. of patients receiving—				Total No. of doses	No. of patients with initial exposure to PMM	Total No. of patients
		1st dose	*2nd dose*	*3rd dose*	*> 3 doses*			
Single dose every 3 wks	80	4	3	1	0	8	4	4
	160	4	2	1	1	8	4	4
	240	3	3	1	0	7	3	3
	400	3	2	2	0	7	3	3
	560	3	2	0	0	6	4	4
	1000	3	3	0	0	6	2	3
	1500	4	2	1	0	7	4	4
	2000	6	1	0	0	7	5	6
		1–3 doses	*4–6 doses*	*7–9 doses*	*> 9 doses*			
Single dose 3 times/wk (M-W-F) × 4 wks	100	9	4	2		38	5	9

TABLE 3.—Nonhematologic toxicity

	Dose (mg/m^2)	No. of patients	No. of patients with—							
			Nausea	Vomiting	Anorexia	Diarrhea	Weight loss (> 2 kg)	Somnolence	Other CNS changes	Diaphoresis
Single dose every 3 wks	80	4	0	0	1	0	1	0	1 depression	
	160	4	1	0	0	0	2	1		
	240	3	1	0	0	0	1	0		
	400	3	3	3	2	0	1	0		
	560	3	2	0	0	0	1	1	1	
	1000	3	3	3	1	1	2	4		1
	1500	4	4	3	3	1	3	3	1 headache, 1 depression	
	2000	6	6	6	4	0	0	4	1 headache, 1 confusion	4
Single dose 3 times/wk (M-W-F) × 4 wks	1000	9	9	9	0	0	0	0	1 visual hallucinations	0

12 patients treated at > 1000 mg/m^2 became drowsy, one became somnolent, two complained of a dull frontal headache, and one became confused. The onset of these toxic effects was at the very end of the PMM infusion and persisted for 30–120 minutes. Fundoscopic changes were not observed in any of these patients exhibiting these effects. Electroencephalography (EEG) was performed on four patients during PMM infusion and for 30 minutes after infusion (one patient at 560 mg/m^2, two at 1500 mg/m^2, and one at 2000 mg/m^2). The EEGs demonstrated diffuse slowing of alpha and beta waves compatible with a metabolic encephalopathy in those patients treated at > 1000 mg/m^2 during the last 15–30 minutes of PMM administration; these toxic effects persisted for 15–30 minutes after infusion. None of these patients had demonstrable CNS lesions or deficits by computerized tomographic (CT) scan or EEG, other than those seen on EEG during PMM administration. Other nonhematologic toxic effects included diaphoresis during PMM administration in six patients (one of four patients at 560 mg/m^2, one of three at 1000 mg/m^2, and four of six at 2000 mg/m^2). These effects were not associated with any changes in vital signs.

Multiple-Dose Schedule (M-W-F)

Nine patients received 38 doses (range, one to nine; median, four) of PMM as a single dose on the M-W-F schedule every week. All patients were evaluable for toxicity. The dose-limiting toxic effects included vomiting and persistent nausea which occurred in all patients. Five patients refused further PMM treatment within 10 days after starting this schedule (one to five doses). Two patients developed cardiac arrhythmias temporarily related to the second dose of PMM and were discontinued from the study. The last patient was able to tolerate 3 weeks (nine doses) of therapy. One patient developed visual hallucinations several hours after each of two treatments with PMM and was discontinued from the study. An EEG and brain CT scan were normal.

No other toxic effects were observed in this study. Specifically, no change was seen in the blood cell count parameters, the serum coagulation, or the biochemical tests of any patient receiving either the single- or multiple-dose schedule while in the study. No alopecia, phlebitis, or acute or allergic side effects were observed in this study.

Therapeutic Response

Minor tumor regressions were seen in two patients. One patient, who had a pleural mesothelioma, had a 25%–50% reduction of a pleural mass as measured by thoracic CT scan after three doses of PMM at 400 mg/m^2. The dose was escalated to 1000 mg/m^2 but the disease progressed after 1 month. The other patient, who had a parotid gland tumor with metastasis to the brain, lungs, and cervical nodes, had received PMM at a dose of 1500 mg/m^2. The CNS and pulmonary metastasis remained stable while the cervical adenopathy decreased by 50% for a period of 4 weeks. The patient's disease then progressed.

DISCUSSION

The results of this study indicate that PMM has dose-limiting and dose-related CNS toxic effects. These effects began as mild nausea and vomiting at

a dose of 400 mg/m² and progressed to confusion, headache, and continuous vomiting for 24 hours at a dose of 2000 mg/m². The choice of an MTD for the single-dose (every 3 weeks) schedule would appear to be 1500 mg/m². Patients are able to tolerate this level because of the intermittent schedule. However, when the dose is decreased to even 1000 mg/m² on a multiple-dose schedule (a dose well-tolerated once every 3 weeks), patients become anorectic, have persistent nausea, and invariably refuse further therapy with PMM after 2–3 weeks. To ensure any kind of patient tolerance to further courses of PMM therapy at 1000 mg/m² on a multiple-dose schedule, it is felt that the maximum number of doses that can be given is three over a period of 1 week (M-W-F) with a 2-week rest being necessary to reverse the anorexia and nausea.

The recommended dose for HMM as a single agent is 300 mg/m²/day × 90 days[5] or 450 mg/m²/day × 21 days. Even with partial absorption and proportional activity the MTD for PMM is probably only a fraction of the MTD for HMM. When considering the multiple-dose PMM schedule, patients were only able to tolerate a median of 2 weeks (6000 mg/m² total dose) of therapy. This is not any different from what could be delivered as oral HMM. Although the dose-response relationship for this drug is unknown, it is possible that the total amount of antineoplastic drug that can be delivered in a 3–4-week interval may be related to an antineoplastic response. If this is true, then it would appear that PMM offers little advantage over HMM and further clinical trials may not be warranted. On the other hand, it is conceivable that higher peak intravascular drug levels may offer a therapeutic advantage; in that case PMM may have some advantage over HMM.

The toxic effects of PMM in this study have some similarities and differences compared to those of HMM. The hematologic toxicity of HMM occurs as early as 1 week after administration of a higher dose intermittent schedule (450 mg/m²/day × 21 days with 4-week rest periods), but does not occur for several months with a low-dose, continuous schedule (300 mg/m²/day) (5). Hematologic toxicity was not seen in this phase I study of PMM even though the patients receiving the multiple-dose schedule were given an amount of drug roughly comparable to the higher dose HMM schedule. The neurologic disturbances of HMM are paresthesias, numbness, sleep disturbances, mental depression, and ataxia. These toxic effects do not develop on the high-dose, intermittent schedule but occur in a small percent of patients on the low-dose, continuous schedule. In this study comparable neurologic toxicity was produced repeatedly with iv PMM, suggesting that peak drug levels are the cause of the CNS disturbances, especially since they are rapidly reversible within 24 hours. It would appear that in some patients receiving HMM there is slow progressive accumulation of drug which eventually precipitates neurologic symptoms.

PMM has dose-limiting neurologic toxicity which precludes administering a large iv dose of PMM. The MTD of PMM given as a single-dose, 2-hour infusion is 1500 mg/m² every 3 weeks. A dose of PMM at 1000 mg/m² given as a single 2-hour infusion three times a week (M-W-F) for 4 weeks is not tolerable, and the MTD for this schedule is 1000 mg/m² given as a 2-hour infusion three times a week (M-W-F) for three doses, with courses repeated every 3 weeks. Other dose schedules may need to be explored in order to maximize the amount of drug administered.

[5]Guidelines for the clinical use of hexamethylmelamine (NSC-13875) for ovarian cancer. Prepared by the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD, Mar 1977.

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High-Dose Cisplatin With Fluid and Mannitol-Induced Diuresis in Advanced Lung Cancer: A Phase II Clinical Trial of the EORTC Lung Cancer Working Party (Belgium)[1,2]

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SUMMARY

A phase II clinical trial of high-dose cisplatin (120 mg/m² iv every 3 weeks), with fluid and mannitol-induced diuresis, was conducted in 81 patients with advanced lung cancer. Partial remissions were documented in 26% of 75 evaluable cases for a median duration of 3.5 months. Adenocarcinoma and small cell anaplastic carcinoma were more responsive than epidermoid carcinoma, with partial response rates of 35%, 30%, and 18%, respectively. The median survival of responders (8.5 months) was significantly longer than the survival of nonresponders (4 months) ($P < 0.02$). Myelosuppression was mild. Renal toxicity with peak serum creatinine > 2.5 mg/100 ml occurred in eight patients, with one death occurring due to toxicity. Cisplatin is an active drug in advanced lung cancer.

[Cancer Treat Rep 64:1341–1346, 1980]

Cisplatin (*cis*-diamminedichloroplatinum[II]), is an inorganic coordination compound of platinum inhibiting DNA synthesis in mammalian cells through cross-linking of DNA strands in a manner similar to bifunctional alkylating agents (1,2).

The antitumor activity of cisplatin in testicular, bladder, cervical, ovarian, and head and neck cancers is well-documented (3,4). Data on lung cancer are few, although single responses have been reported in some series using doses of 50–75 mg/m² iv (6–8). In addition, the demonstration of the therapeutic activity of several combination chemotherapy regimens, including cisplatin, in small cell and non-small cell lung cancer (9–25) suggested the necessity of reassessing the role of cisplatin alone in these diseases.

The dose-limiting nephrotoxicity of cisplatin can be decreased by prehydration and mannitol-induced osmotic diuresis, allowing the use of high doses (100–120 mg/m²) in clinical trials. Data suggest that these high doses have resulted in an increased response rate in testicular cancer (5).

Since no clinical trial of high-dose cisplatin in lung cancer had been reported, such a study was initiated within the European Organization for Research on Treatment of Cancer (EORTC) Lung Working Party on July 1, 1978, and terminated on September 1, 1979.

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METHODS

Patient Eligibility

All patients had advanced biopsy-proven bronchogenic carcinoma. Two pathologists independently reviewed the slides submitted by participating institutions and classified each case according to World Health Organization nomenclature (26). Most patients with epidermoid carcinomas, adenocarcinomas, and large cell carcinomas were previously untreated with chemotherapy, and ten of 13 patients with small cell anaplastic carcinoma were given cisplatin as second- or third-line treatment after two to four drug combinations of CCNU, cyclophosphamide, vincristine, methotrexate, and doxorubicin (adriamycin). Cisplatin was not administered within 4 weeks of prior chemotherapy.

None of the patients were candidates for potentially curative surgery or radiation therapy. All had measurable or evaluable lesions with documented progression prior to entry into the study. Previously irradiated lesions and pleural effusions were not accepted as criteria for evaluation. Bone metastases were accepted only if measurable disease was present elsewhere.

Patients with more than one primary tumor were excluded. The following additional requirements had to be met: a Karnofsky performance status of ≥ 50, a serum creatinine < 1.5 mg/100 ml and/or a creatinine clearance ≥ 80 ml/minute, a wbc count $> 3000/mm^3$, and a platelet count $> 100,000/mm^3$.

Treatment

Cisplatin[4] (120 mg/m^2) was administered iv for 15 minutes with mannitol-induced diuresis, according to the method of Hayes et al (5), every 3 weeks until progression of the disease.

The method of hydration consisted of giving 1000 ml of 5% dextrose in 0.45% NaCl for 4 hours before cisplatin was given and 4000 ml of 5% dextrose in 0.45% NaCl with 20 mEq K^+/liter for the next 24 hours.

Mannitol (12.5 mg) was injected as an iv bolus immediately prior to giving cisplatin and as a continuous 20% solution (60 ml/hour) for the next 6 hours.

Diuresis and emesis were measured every hour for the first 6 hours and every 6 hours up to 24 hours thereafter. If urine output decreased to < 75 ml/hour, 80 mg of furosemide was given iv.

For nausea and vomiting, metoclopramide (10 mg iv) was given every 3 hours as needed. When this was insufficient, a single dose of haloperidol (1 mg im) was added.

Serum creatinine values were determined before, 24 hours after, and/or 48 hours after each cisplatin treatment. If transient elevation of 1.5–2.5 mg/100 ml occurred, the next dose was reduced by 50%. If an elevation > 2.5 mg/100 ml occurred, cisplatin treatment was discontinued. None of the patients received aminoglycosides to treat infectious complications of their disease.

Treatment was delayed by 1 week if wbc count remained $< 4000/mm^3$ or platelet count was $< 150,000/mm^3$ 3 weeks after cisplatin.

Treatment Evaluation

Baseline studies of extent of disease before initiating cisplatin treatment consisted of: chest roentgenogram, lung tomograms, and fibrobronchoscopy; bone scan with roentgenograms of symptomatic areas or to confirm bone scan abnormalities; liver scan; brain scan or computerized tomography (CT) (mandatory for small cell anaplastic carcinoma only); and minimal laboratory studies, including cbc, serum creatinine and/or creatinine clearance, liver function tests, and urinalysis. Audiometry was optional.

Followup every 3 weeks included checking the clinical status of the patient, recording symptoms, drug side effects, Karnofsky performance status, and weight and tumor measurements and obtaining chest roentgenograms and repeated minimal laboratory studies.

At 6 weeks, 3 months, and every 3 months thereafter, fibrobronchoscopy, lung tomograms, and radioisotopic scans were repeated to evaluate the therapeutic response.

Patients who received only one dose of cisplatin because of early death or severe toxicity were considered as evaluable cases. The duration of response is the period between starting treatment and progression of disease. Therapeutic responses were defined as follows: *complete remission*—disappearance of all symptoms and signs of disease for at least 4 weeks; *partial remission*—a decrease $\geq 50\%$ in the product of the two largest perpendicular diameters in all measurable lesions, with no lesions developing for at least 4 weeks, or unequivocal regression of evaluable but nonmeasurable lesions, agreed on by at least two investigators; *stable disease*—$< 50\%$ decrease and $< 25\%$ increase in the product of the two largest perpendicular diameters of all measurable lesions for at least 4 weeks; *disease progression*—$> 25\%$ increase in the size of any lesion or appearance of new lesions; *early death*—

[4]Cisplatin was supplied by the Bristol-Myers Co.

death due to progression of disease within 3 weeks of starting treatment; and *toxic death*—death occurring because of drug toxicity.

Each case entered in the study was presented at one of the monthly meetings of the EORTC Lung Cancer Working Party at the time of registration, evaluation, and end of treatment. Remission was accepted on presentation of supportive evidence. Less than a partial remission was not considered as objective response.

The evaluation of pulmonary lesions was particularly stringent: only patients with clearly evaluable lesions were admitted to the study. Improvement in lung atelectasis was not accepted as evidence of tumor regression. Tumor response was documented with chest roentgenograms, tomograms, and bronchoscopy.

TABLE 1.—Patient characteristics

No. of patients adequately treated/No. entered	75/81
Median age in yrs (range)	60 (35–77)
Sex (males/females)	76/5
Median initial Karnofsky performance status (range)	70 (50–100)
No. of patients with—	
Prior therapy	
Chemotherapy only	10
Radiotherapy	20
Chemotherapy and radiotherapy	8
None	43
Histologic classification	
Epidermoid carcinoma	43
Adenocarcinoma	22
Large cell carcinoma	2
Small cell anaplastic carcinoma	13
Mixed type	1

RESULTS

Therapeutic Response

The characteristics of the patient population are given in table 1. High-dose cisplatin treatment resulted in an overall 26% partial response rate in 75 evaluable patients with advanced lung cancer (table 2). Six cases were not evaluable because of loss to followup (four) or inadequate criteria for evaluation (two).

Partial remissions were seen in all cell types, but the response rate was higher in adenocarcinoma (35%) and small cell carcinoma (30%) than in epidermoid carcinoma (18%). There were only two patients with large cell carcinoma, but one did show a partial response.

Three patients with small cell carcinoma had not received prior chemotherapy. In the epidermoid carcinoma group, seven of 24 previously untreated patients responded. None of 15 patients with prior radiation therapy (11) and chemotherapy (four) responded. A similar trend was not found in adenocarcinoma: four of nine patients without prior treatment and three of 11 with prior radiation and/or chemotherapy responded. However, none of the four patients with adenocarcinoma who had previously received both radiation and chemotherapy responded. Tumor regressions were documented in all metastatic sites except the brain. Recalcification of a lytic bone metastasis occurred in one case.

The median duration of response was 3.5 months (range, 1.5–8 months). Excluding eight patients with early death, the median survival of the remaining 67 patients was 5 months. The actuarial survival curves for responders and nonresponders (fig 1) were compared using the Wilcoxon and log-rank tests: $P \leq 0.05$ was considered to be statistically significant. The median survival of the 20 responders (8.5 months) was significantly longer than the survival of nonresponders (4 months) ($P < 0.01$). These two groups were comparable in terms of the initial Karnofsky performance status. By cell type, however, the difference in survival of responders and nonresponders was only significant for epidermoid carcinomas ($P < 0.02$). For adenocarcinomas and small cell carcinomas, statistical significance was not achieved, possibly because there were too few patients.

TABLE 2.—Therapeutic activity

Histology	No. of patients						Partial remission
	Entered	Evaluable	Partial remission	Stable disease	Disease progression	Early deaths	
Epidermoid cancer	43	39	7	7	19	6	18%
Adenocarcinoma	22	20	7	6	7	0	35%
Large cell cancer	2	2	1	0	1	0	1 of 2 patients
Small cell anaplastic cancer	13	13	4	0	7	2	30%
Mixed type cancer	1	1	1	0	0	0	1 of 1 patient
Total	81	75	20	13	34	8	26%

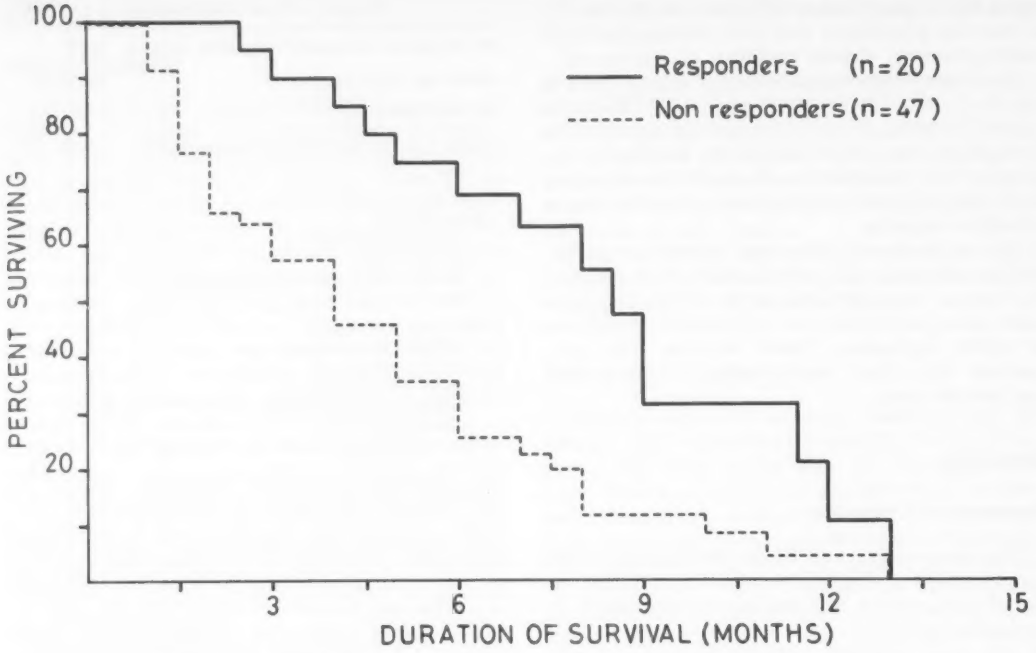

FIGURE 1.—Actuarial curves for survival of responders and nonresponders receiving cisplatin.

Toxicity

The median number of doses administered was two per patient, but responding patients received a median of four doses (range, three to seven). Seventy patients were available for toxicity analysis after the first dose of cisplatin (table 3); 11 did not receive a second dose because of renal toxicity (eight), prolonged thrombocytopenia (two), or severe vomiting (one).

Nearly all patients experienced nausea and vomiting, which were severe in about one third. Myelosuppression was mild, but four patients had profound thrombocytopenia; there was no drug-related sepsis or hemorrhage.

Renal toxicity (peak serum creatinine > 1.5 mg/100 ml) occurred in 12 cases (17%). Eight patients with peak serum creatinine > 2.5 mg/100 ml did not receive a second dose. Two patients developed severe renal insufficiency with a serum creatinine > 5.0 mg/100 ml. One recovered after peritoneal dialysis, and the other died from drug-related complications. It appeared that cisplatin was the only contributing factor in the patients who exhibited renal function impairment. Aminoglycosides and other nephrotoxic drugs were avoided in this study.

Six of 12 patients who developed renal toxic effects had normal serum creatinine levels 24 hours after cisplatin administration. There was no evidence of cumulative toxicity, but the number of patients receiving three or more doses was small. Only one of 20 responding patients had treatment discontinued after three doses because of a serum creatinine of 2.5 mg/100 ml.

Loss of hearing was clinically significant in one patient. Routine audiometric studies were not performed.

Abnormal liver function tests and gynecomastia were considered as toxic side effects in two cases, but the interpretation of those observations is difficult.

No alopecia or peripheral neuropathy was recorded. Hypomagnesemia was not evaluated because serum magnesium levels were not routinely obtained in this study.

DISCUSSION

Contrary to the opinion expressed in a recent review (4), published data do not support the lack of activity of cisplatin in lung cancer. Early phase

TABLE 3.—Toxic effects in 70 evaluable cases (1st course)

Effect	No. of patients
Nausea and vomiting	Nearly all
Hematologic toxicity	
Hemoglobin decrease > 2 mg/100 ml	10
Wbc count nadir (cells/mm³)	
< 4000	12
< 1500	—
Platelet count nadir (cells/mm³)	
< 100,000	7
< 20,000	4
Renal toxicity	12
Peak serum creatinine (mg/100 ml)	
1.6–2.5	4
2.6–5.0	6
⩾ 5.1	2*
Ototoxicity (clinical)	1
Abnormal liver function	1
Gynecomastia	1

*1 toxic death.

II trials and more recent studies have documented objective tumor regressions with cisplatin alone (6–8,27) or in combination with other chemotherapeutic agents (11–25) in small cell and non-small cell lung cancers. These results suggesting therapeutic activity made it desirable to reassess the value of cisplatin as a single agent. The 26% response rate in 75 evaluable patients in the present study demonstrates that cisplatin is an active drug in the treatment of lung cancer.

Adenocarcinoma and small cell anaplastic carcinomas appear to be the most sensitive cell types. These results add support to the data of Eagan et al (9) on combination chemotherapy of cyclophosphamide, doxorubicin, and cisplatin in adenocarcinoma and of Sierocki et al (10) on VP-16-213 and cisplatin in small cell anaplastic carcinoma.

Epidermoid carcinomas appear less responsive, but clinically useful objective tumor regressions were documented in this cell type. All partial remissions occurred in patients who were not previously treated with radiation or chemotherapy, which stresses the importance of patient selection in detecting therapeutic activity in this disease.

Although nausea was sometimes severe, toxic effects were acceptable to the majority of patients. The single toxic death occurred in an individual with normal renal function.

This study demonstrates a significant therapeutic activity of high-dose intermittent cisplatin given as a single agent with fluid- and mannitol-induced diuresis to patients with advanced lung cancer. Therefore, the drug should continue to be investigated as part of combination chemotherapy.

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Biochemical Approaches to Enhancement of Antitumor Drug Selectivity: Selective Protection of Cells From 6-Thioguanine and 6-Mercaptopurine by Adenosine[1,2]

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SUMMARY

The cytotoxicity of 6-thioguanine and 6-mercaptopurine to cultured lymphoblasts and fibroblasts was strongly antagonized by pretreatment of the cells with 100 μM adenosine. Administration of adenosine 2 hours after the antipurine agent did not cause antagonism. In two rat hepatoma cell lines, adenosine pretreatment did not protect cells from the antipurines. Treatment of lymphoblasts or fibroblasts with 100 μM adenosine gave increases up to 150% in cellular ATP and ADP and decreases > 80% in UTP and UDP. In the hepatoma lines, adenine nucleotides did not increase by > 45%, and uridine nucleotides did not decrease by > 40% following adenosine treatment. The selective protection of the normal cells from 6-thioguanine and 6-mercaptopurine was probably the consequence of phosphoribosylpyrophosphate (PRPP) depletion, since adenosine pretreatment decreased PRPP pools by > 90% in the normal cells but by only 30% in the malignant hepatoma cells. In the absence of PRPP the antipurines would not be metabolically activated. The selectivity of the adenosine and antipurine combinations was probably attributable to the low activity of adenosine kinase and high activities of adenosine deaminase and PRPP synthetase characteristic of malignant hepatomas.

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Adenosine is toxic to mammalian cells in culture (1), and the cytotoxicity of adenosine to fibroblasts was shown to be reversible by uridine (2). It was thus suggested that the primary lesion caused by adenosine was depletion of cellular phosphoribosylpyrophosphate (PRPP) (2). The de novo biosynthesis of both purines and pyrimidines requires PRPP; in the presence of adenosine the cells have an adequate supply of purines, and thus the biosynthesis of UMP becomes rate-limiting for cell growth. Adenosine treatment causes an overshoot of cellular ATP and ADP concentrations (3) and these nucleotides are known to be feedback inhibitors of PRPP synthetase (4). An alternative explanation of the inhibition of PRPP synthetase by adenosine was that the conversion of adenosine to its nucleotides might deplete the cellular pool of inorganic phosphate, which is an obligatory activator of PRPP synthetase (5).

In comparison with other cell types, malignant hepatoma is rather resistant to adenosine, though toxicity is seen at high doses (6). This observation may be explained by the high activity of adenosine deaminase and the low activity of adenosine kinase in hepatomas of rapid growth rate (6). The effect of these enzymatic changes is that proportionately less adenosine is converted into adenine nucleotides (believed to be the inhibitory species) and propor-

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tionately more adenosine is deaminated to its nontoxic metabolite, inosine. In addition to the alterations in activity of adenosine deaminase and adenosine kinase, hepatomas contain abnormally high activity of PRPP synthetase (7). This should presumably increase the cellular potential for PRPP production and may be a third factor that contributes to the adenosine resistance of hepatoma cells.

The antipurine drugs 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP) are metabolized to their active nucleotide forms through the enzyme hypoxanthine-guanine phosphoribosyltransferase in a PRPP-dependent reaction (8,9). The fact that normal cell types were much more responsive to adenosine than hepatoma cells suggested the possibility of selectively protecting normal cells from the cytotoxicity of 6-MP and 6-TG without diminishing the antitumor effect exerted on the hepatoma cells.

MATERIALS AND METHODS

6-TG, 6-MP, adenosine, hypoxanthine, and PRPP were purchased from Sigma Chemical Co, St. Louis, MO. [^{14}C]hypoxanthine was obtained from Amersham/Searle Corp, Arlington Heights, IL. High-pressure liquid chromatography columns were purchased from Whatman, Inc, Fair Lawn, NJ, and thin-layer chromatography plates coated with polyethyleneimine cellulose were from Macherey-Nagel and Co, Düren, W Germany. Tissue culture supplies were from Grand Island Biological Co, NY.

Cell cultures.—All cell lines were maintained in McCoy's medium 5A supplemented with 5% dialyzed horse serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). For growth inhibition experiments, 10-ml cultures were initiated in plastic flasks (Falcon Plastics, Oxnard, CA) at a cell density of 3×10^4/ml. Novikoff hepatoma cells of line N_1S_1-67 (10) were grown in stationary suspension culture; their mean log-phase doubling time under these conditions was 11 hours. Morris hepatoma cells of line 3924A (11) were grown in monolayer culture, with a mean log-phase doubling time of 15 hours. W1-L2 lymphoblasts (12) were grown in stationary suspension culture and had a doubling time of 19 hours. BF5 is a rat fibroblast line initiated from skin of the BUF rat. It grew in monolayers with a mean doubling time of 28 hours.

Ribonucleotide analysis.—About 2×10^6 cells were harvested by centrifugation at $1500 \times g$ for 5 minutes, and cell pellets were extracted in 1 ml of ice-cold 0.7 N perchloric acid. Extracts were centrifuged at $20,000 \times g$ for 20 minutes to remove precipitated protein. Perchlorate was removed from the supernatant by addition of 75 mg of potassium bicarbonate, followed by centrifugation at $1500 \times g$ for 5 minutes. The extracts were then brought to pH 2.6 by addition of 25 μl 4 N HCl. Aliquots (200-μl) were analyzed for ribonucleotides by high-pressure liquid chromatography on a Whatman Partisil PSX10/25 SAX strong anion-exchange column (25 × 0.46 cm) using buffer conditions described by Lui et al (13). 6-Thioinosinate (6-thioIMP) was measured on the same column using variable wavelength detector tracking at 320 nm; the physiologic nucleotides do not absorb at this wavelength.

PRPP assays.—About 5×10^7 cells were harvested by centrifugation at $1500 \times g$ for 5 minutes, and 0.6 ml of extraction medium consisting of 50 mM potassium phosphate buffer (pH 7.5) containing 0.25 M sucrose and 0.1 mM disodium EDTA was added to the cell pellet. Cells were lysed by freezing the suspension in liquid nitrogen, thawing, and then boiling for 20 seconds. Lysates were cooled on ice and centrifuged for 30 minutes at $100,000 \times g$. Assays were then run immediately. The following was added to a microfuge tube: 90 μl of triethanolamine HCl buffer (pH 7.8, 0.4 M); 10 μl of $MgCl_2$ (100 mM); 25 μl of [^{14}C]hypoxanthine (0.8 mM; 20 microcuries/ml; 25 μl of hypoxanthine-guanine phosphoribosyltransferase (0.8 IU/ml, purified from human erythrocytes by the method of Krenitsky et al [14]); and, finally, 150 μl of the cell extract. Blank tubes were set up using extraction buffer instead of extract, and standards with 2.5–15 nmols of PRPP. Tubes were incubated at 37°C for 30 minutes, and 25-μl aliquots were spotted on 2-cm squares of polyethyleneimine cellulose thin-layer plate. These squares were washed three times in 600 ml of 1 mM sodium formate and once in 95% ethanol. They were then dried and counted in a scintillation counter. Counting efficiency was determined using unwashed squares. This procedure gave a linear response for up to 15 nmols of PRPP.

RESULTS

Figure 1 shows dose-response curves of the four cell lines to adenosine. The two hepatoma lines were about tenfold more resistant to adenosine than the fibroblasts and lymphoblasts. In the experiments shown in figure 1, fresh adenosine-containing medium was added after 24 and 48 hours. A single adenosine administration caused a transient inhibition from which cells subsequently partially recovered (table 1). Adenosine toxicity is dependent on the inoculum density of the cultures, and in order to minimize the lethal effect of adenosine to normal cells, a comparatively high inoculum density (3–3.5 $\times 10^4$ cells/ml) was used in the present study.

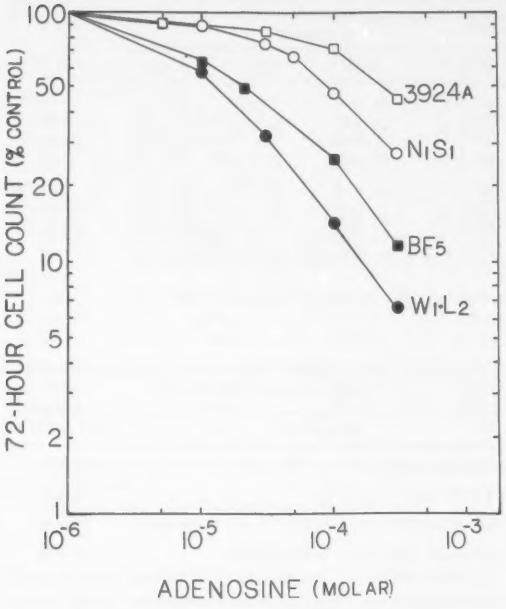

FIGURE 1.—Dose-response curves for growth inhibition of the 4 cell lines by adenosine (log-log plots). Duration of treatment was 72 hrs, and media were changed after 24 and 48 hrs. Growth conditions were as detailed in the Materials and Methods section.

Dose-response curves for 6-TG and 6-MP are shown in figures 2 and 3, respectively. The hepatoma cells were comparatively sensitive to these antipurine agents, perhaps because of the high PRPP synthetase activity of these cells.

If cells were treated with adenosine 2 hours before administration of 6-TG and 6-MP, some degree of antagonism was observed in all the cell lines. An adenosine concentration of 100 μM was selected for detailed study since, at this level, the protection of nonmalignant cells from 6-TG or 6-MP was maximal (without the toxicity from adenosine being lethal) and because, at this concentration, the 6-MP and 6-TG effects were not strongly antagonized in the hepatoma cells. Results of growth inhibition experiments are summarized in table 1; 10 μM 6-TG or 80μM 6-MP, preceded by 100 μM adenosine, had considerable selectivity against the hepatoma cells.

Table 2 shows data that indicate the time-sequence dependence for the adenosine and 6-TG combination in W1-L2 cells. Simultaneous administration of the two agents resulted in partial antagonism of the cytotoxic effect of 6-TG, but the antagonism was most pronounced when adenosine preceded the 6-TG. When adenosine was added 2 hours after 6-TG, no antagonism was seen, and the combination gave partially additive results; ie, growth inhibition was greater than that produced by 6-TG alone. This time-sequence dependence suggested that adenosine was blocking the PRPP-dependent activation of the 6-TG.

Results of adenosine treatment on the ribonucleotide pools of the four cell lines are listed in table 3. In the nonmalignant cell lines (BF5 and W1-L2), large increases were seen in ATP and ADP and marked decreases were seen in the pyrimidine nucleotides. The effects of adenosine in the hepatoma cells were qualitatively similar but much less extensive. However, there is some indication that the mechanism of adenosine toxicity in hepatomas may differ from that in other cells; eg, in hepatoma 3924A, deletion of adenosine kinase does not confer resistance to adenosine (D. P. Suttle and R. C. Jackson, unpublished observation). Further evidence for an adenosine kinase-independent mechanism of adenosine toxicity in hepatomas is given in table 4, which shows that deoxyadenosine treatment produced a decrease in UTP and UDP pools similar to that given by adenosine. Deoxyadenosine is not phosphorylated by adenosine kinase in these cells

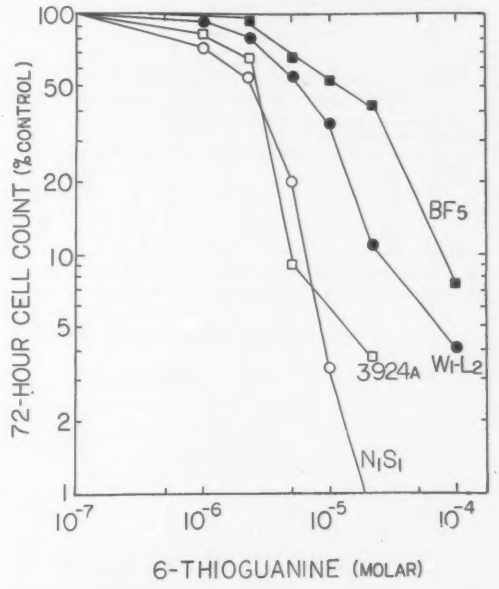

FIGURE 2.—Dose-response curves for growth inhibition of the 4 cell lines by 6-TG (log-log plots). Duration of treatment was 72 hrs, and growth conditions were as described in the Materials and Methods section.

TABLE 1.—Effect of pretreatment with adenosine on cytotoxicity of 6-TG and 6-MP in vitro

Treatment	Cell counts*			
	W1-L2	BF5	3924A	N_1S_1
Adenosine (100 μM)	64 ± 6	73 ± 5	70 ± 7	72 ± 6
6-TG (10 μM)	36 ± 3	53 ± 4	11 ± 2	8 ± 2
Adenosine (100 μM) + 6-TG (10 μM) 2 hrs later	83 ± 5†	79 ± 4†	17 ± 4	13 ± 3
6-MP (80 μM)	39 ± 4	41 ± 3	18 ± 2	28 ± 4
Adenosine (100 μM) + 6-MP (80 μM) 2 hrs later	96 ± 3†	67 ± 4†	22 ± 3	31 ± 2

*Values = mean ± SEM for triplicate cultures, expressed as % of untreated control cultures after 72 hrs of growth in continuous presence of the drugs.

†Significant protection when compared with groups treated with the antipurine agent (6-MP or 6-TG) alone ($P < 0.05$, 2-tailed Student's t test).

(there is a separate deoxyadenosine kinase), and deoxyadenosine did not increase the ATP and ADP pools, which were in fact slightly decreased.

The effects of adenosine on cellular pools of uridine nucleotides probably give an indirect indication of the response of the PRPP pool. Table 5 shows results of direct measurements of the cellular PRPP pool in the four cell lines. In the nonmalignant BF5 and W1-L2 cells PRPP was substantially depleted following treatment with 100 μM adenosine, whereas in the hepatoma lines the depletion was only about 30%.

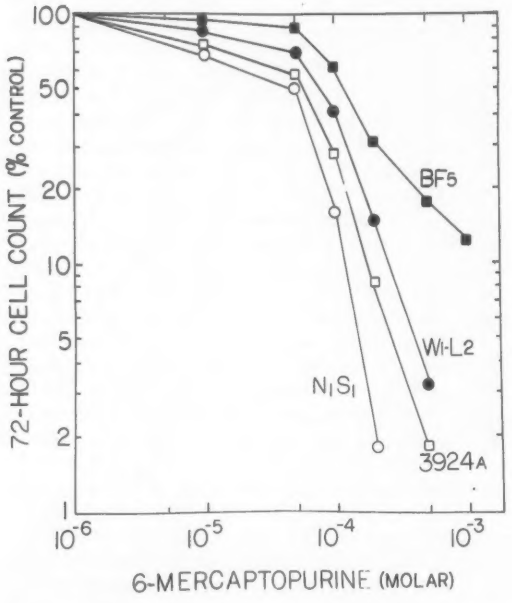

FIGURE 3.—Dose-response curves for growth inhibition of the 4 cell lines by 6-MP (log-log plots). Duration of treatment was 72 hrs, and growth conditions were as described in the Materials and Methods section.

TABLE 2.—Time-sequence dependence of adenosine-6-TG antagonism in W1-L2 cells

Treatment	Cell count*
Adenosine (100 μM)	69 ± 7
6-TG (10 μM)	38 ± 3
Adenosine (100 μM) + 6-TG (10 μM) 2 hrs later	75 ± 8†
Adenosine (100 μM) + 6-TG (10 μM), simultaneously	55 ± 6
6-TG (10 μM) + adenosine (100 μM) 2 hrs later	32 ± 4

*Values = mean ± SEM of triplicate cultures, expressed as % of untreated control cultures, after 72 hrs of growth in continuous presence of the drugs.

†Significant protection when compared with the group treated with 6-TG alone ($P < 0.05$, 2-tailed Student's t test).

Table 6 shows the effect of adenosine pretreatment on the formation of 6-thioIMP, the active metabolite of 6-MP, during a 3-hour incubation with 80 μM 6-MP. In the lymphoblasts and fibroblasts the PRPP-dependent metabolic activation of 6-MP was almost eliminated, while only a minor decrease was observed in 6-thioIMP accumulation in the hepatoma lines. No diphosphate or triphosphate nucleotides of 6-MP were observed in any of the samples. In all four cell lines, 6-MP caused a minor decrease in ATP pools, and an extensive decrease in GTP. In the normal cells adenosine blocked both these effects of 6-MP. In the hepatoma cells adenosine prevented the minor effect of 6-MP on ATP pools, but the major cytotoxic effect of 6-MP, the inhibition of guanylate nucleotides, was not blocked (table 6).

A further difference in the response of hepatoma cells to adenosine from that of other cell lines is seen in the ability of uridine to reverse the adenosine effect. In the fibroblasts and lymphoblasts ur-

TABLE 3.—Cellular ribonucleotides after adenosine treatment*

Cell line	Ribonucleotides (nmols/10^9 cells)†						
	ATP	ADP	GTP	GDP	UTP	UDP	CTP
W1-L2							
Control	2350	1220	1060	590	1970	840	65
Treated	5520	2250	750	530	390	150	10
%	235	184	71	90	20	18	15
BF5							
Control	10,050	3210	2560	1230	2440	880	290
Treated	25,020	6320	4920	1820	410	170	93
%	249	197	192	148	17	19	32
3924A							
Control	4260	1050	1030	380	1400	270	580
Treated	5880	1480	950	420	910	220	540
%	138	141	92	110	65	80	79
N_1S_1							
Control	5990	1270	1620	380	850	240	330
Treated	8690	1820	1590	460	500	150	290
%	145	143	98	120	59	63	88

*Cells were treated for 16 hrs in 100 μM adenosine, and nucleotides were extracted and assayed as described in the Materials and Methods section.

†Values = mean of triplicate determinations.

idine almost completely reversed the adenosine-induced growth inhibition (table 7), as previously reported (2). Hepatoma cells, in contrast, were protected from adenosine by uridine only to a comparatively minor extent. This observation suggested that the selectivity of the adenosine and 6-TG combination could be further enhanced by uridine. Preliminary results in the W1-L2 and 3924A lines are shown in table 8. Addition of uridine to the protocol reduced the toxicity of adenosine to the nonmalignant W1-L2 cells, without significantly decreasing the efficacy of the adenosine and 6-TG combination in the malignant hepatoma 3924A cells.

DISCUSSION

The limited selectivity of anticancer drugs is usually attributed to the absence of exploitable qualitative biochemical differences between cells of tumor and host. Nevertheless, tumors appear to possess an overall pattern of enzyme activities that is distinguishable from the nonmalignant state. A number of attempts to target cytotoxicity selec-

TABLE 4.—Effect of deoxyadenosine treatment on ribonucleotide pools in N_1S_1 hepatoma cells*

Nucleotide	% of untreated control†
ATP + ADP	78
GTP + GDP	81
UTP + UDP	48
CTP	23

*Cells were treated for 16 hrs in 100 μM deoxyadenosine, and nucleotides were extracted and assayed as described in the Materials and Methods section.

†Values = mean of triplicate determinations.

TABLE 5.—Cellular pools of PRPP after adenosine treatment*

Cell line	PRPP pool (nmols/10^9 cells)†		Treated as % of control
	Control	Treated	
W1-L2	19 ± 3	1.5 ± 0.5‡	8
BF5	67 ± 5	9 ± 2‡	13
3924A	40 ± 4	29 ± 5	73
N_1S_1	43 ± 3	29 ± 3‡	67

*Cells were treated for 16 hrs in 100 μM adenosine, and PRPP was extracted and assayed as described in the Materials and Methods section.

†Values = mean ± SEM for triplicate cultures.

‡Significantly different from untreated control ($P < 0.05$, 2-tailed Student's t test).

TABLE 6.—Ribonucleotide pools in cells treated with 6-MP and adenosine*

Cell line	Pretreatment	6-thioIMP (nmols/10^9 cells)†	Cellular ribonucleoside triphosphates (% of control pool sizes in cultures not receiving 6-MP)†			
			ATP	GTP	UTP	CTP
W1-L2	None	48 ± 6	91 ± 13	35 ± 3	105 ± 4	83 ± 19
	Adenosine	3 ± 1	208 ± 29	92 ± 10	24 ± 4	18 ± 4
BF5	None	94 ± 12	92 ± 19	25 ± 3	99 ± 11	94 ± 21
	Adenosine	2 ± 1	180 ± 4	102 ± 7	32 ± 2	35 ± 5
3924A	None	174 ± 16	80 ± 5	21 ± 3	103 ± 9	89 ± 11
	Adenosine	137 ± 15	139 ± 10	24 ± 2	70 ± 6	85 ± 5
N_1S_1	None	190 ± 18	76 ± 5	17 ± 1	110 ± 10	85 ± 3
	Adenosine	106 ± 22	142 ± 8	26 ± 1	74 ± 4	63 ± 4

*Treatment was with 80 μM 6-MP for 3 hrs, preceded where indicated by 100 μM adenosine for 2 hrs.
†Values = mean ± SEM for triplicate cultures.

TABLE 7.—Reversal of adenosine toxicity by uridine

Treatment	Cell counts*			
	W1-L2	BF5	3924A	N_1S_1
Adenosine (100 μM)	44 ± 4	53 ± 3	68 ± 5	62 ± 3
Uridine (100 μM)	86 ± 6	97 ± 11	95 ± 4	103 ± 9
Adenosine (100 μM) + uridine (100 μM)	83 ± 7†	95 ± 8†	70 ± 5	65 ± 6

*Values = mean ± SEM for triplicate cultures, expressed as % of untreated control cell counts after 72 hrs of growth in continuous presence of the nucleosides.
†Significantly different from group treated with adenosine alone ($P < 0.05$, 2-tailed Student's t test).

TABLE 8.—Effect of pretreatment with adenosine + uridine on cytotoxicity of 6-TG in vitro

Treatment	Cell counts*	
	W1-L2	3924A
Adenosine (100 μM)	53 ± 4	66 ± 6
6-TG (10 μM)	39 ± 3	14 ± 2
Adenosine (100 μM) + 6-TG (10 μM) 2 hrs later	67 ± 5†	17 ± 3
Adenosine (100 μM) + uridine (100 μM) + 6-TG (10 μM) 2 hrs later	88 ± 4†	19 ± 2

*Values = mean ± SEM for triplicate cultures, expressed as % of untreated control cell counts after 72 hrs of growth in continuous presence of the drugs.
†Significant protection when compared with groups treated with 6-TG alone ($P < 0.05$, 2-tailed Student's t test).

tively against cells with particular enzyme patterns have been described. One such approach involved selective "trapping" (depletion) of key metabolites (11,13), and another method of increasing drug selectivity is the technique of selective rescue (15); an example is the use of thymidine for selective rescue of normal cells from an otherwise lethal concentration of methotrexate (16). The results described in the present report represent another approach to enhanced antitumor drug selectivity, ie, selective protection. In normal cells, adenosine causes depletion of cellular PRPP; hepatoma cells deaminate proportionately more adenosine, phosphorylate less, and in addition have an unusually active PRPP synthetase (6,7). The basic idea of the present study was that adenosine, by depleting PRPP in normal cells, might protect them from 6-TG and 6-MP without decreasing the cytotoxic effect against the hepatoma cells. The results in table 1 show that this strategy was partially successful. The time-sequence dependence of the effect (table 2) supports the suggestion that adenosine blocked conversion of 6-TG to its active nucleotide form in lymphoblastic cells. That this was due to PRPP depletion is suggested indirectly by the data in table 3, showing that adenosine caused marked inhibition of UTP biosynthesis in nonmalignant cells, presumably at the PRPP-dependent orotate phosphoribosyltransferase step, while the effect in hepatoma cells was much less marked. Direct evidence for a selective effect of adenosine on PRPP pools is given in table 5, which shows that adenosine caused decreases in PRPP to < 15% of control values in lymphoblasts and fibroblasts, whereas the PRPP remained at 70% of control values following adenosine treatment of hepatomas.

A number of observations concerning the response of hepatoma cells to adenosine cannot yet be satisfactorily explained. The fact that deoxyadenosine caused a drop in cellular UTP without increasing the adenine nucleotide pools (table 4) is a case in point. Another anomalous fact is the lack of reversal of the adenosine effect by uridine in hepatoma cells. However, as table 8 shows, this situation, though still unexplained, may be exploited to increase still further the antihepatoma selectivity of the adenosine and 6-TG combination.

The use of adenosine to enhance selectivity of 6-TG and 6-MP was suggested by the observation of Debatisse and Buttin (17) that fibroblasts could be protected from 8-azaguanine by adenosine plus coformycin. Our observation that normal cells may be protected in this way to a greater extent than malignant hepatoma cells provides a new instance of selective toxicity based on the study of normal and tumor cell enzyme patterns.

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Randomized Clinical Trial Comparing Two Dose Regimens of ICRF-159 in Refractory Malignant Lymphomas[1,2]

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SUMMARY

A clinical trial of ICRF-159 was done in patients with Hodgkin's disease and non-Hodgkin's lymphomas whose tumors had become resistant to conventional chemotherapy. Antitumor effect and toxicity were compared in patients who were randomly assigned to receive either a loading course or a weekly regimen. Among 82 evaluable cases, five of 39 (13%) treated with the loading course schedule and six of 43 (14%) treated with the weekly schedule experienced objective tumor regressions. Response duration tended to be brief (median, 7 weeks). Life-threatening myelosuppression was more frequent in patients receiving the loading course regimen. Survival was somewhat longer among patients receiving the weekly schedule (median survival, 24 versus 12 weeks; $P = 0.04$). ICRF-159 demonstrated definite but limited therapeutic activity in this population of patients with advanced malignant lymphomas. The weekly schedule was associated with fewer episodes of life-threatening toxicity and longer patient survival.

[Cancer Treat Rep 64:1355–1358, 1980]

ICRF-159 is an orally administered agent which has shown significant antitumor activity in a variety of animal tumor systems (1). This compound appears to inhibit DNA metabolism in the G_2-M phase of cell cycle growth, and has lacked cross-resistance with other anticancer agents. Eight of 27 patients with advanced non-Hodgkin's lymphomas treated with a weekly dose regimen by the Western Cancer Study Group (WCSG) experienced objective tumor responses, with five patients having responses lasting > 1 year (2). Experience with a loading course regimen in patients with colorectal cancer at the Mayo Clinic indicated tolerable toxicity and objective responses (3).

The Eastern Cooperative Oncology Group (ECOG) carried out the present investigation (protocol EST 1477) to confirm the previously reported antitumor activity of ICRF-159 in patients with advanced

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: Mayo Clinic, Rochester, MN (*M. J. O'Connell* and *M. N. Silverstein*, CA-13650); Sidney Farber Cancer Institute, Boston, MA (*C. B. Begg*, CA-23318); Hospital of the University of Pennsylvania, Philadelphia (*J. H. Glick*, CA-15488); University of Minnesota, Minneapolis (*M. M. Oken*, CA-20365); Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadephia, PA (CA-18281); Baystate Medical Center, Springfield, MA (CA-20182); Hahnemann Medical College, Philadelphia, PA (CA-13611); Harbor General Hospital, Torrance, CA (CA-21091); Jefferson Medical College, Philadelphia, PA (CA-14215); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); New York University Medical Center, New York (CA-16395); University of Ottawa, Ontario, Canada; University of Montreal Affiliated Hospitals, Quebec, Canada (CA-21653); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); University of Pretoria, South Africa (CA-21692); State University of New York, Stony Brook (Veterans Administration Hospital), Northport (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Wisconsin Clinical Cancer Center, Madison (CA-21115); and Yale School of Medicine, New Haven, CT (CA-15485).

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malignant lymphomas, since this drug could be a useful agent in developing non-cross-resistant combination chemotherapy regimens for earlier clinical application. We elected to compare a weekly versus a loading course administration schedule to determine any advantage in terms of antitumor effect or toxicity.

PATIENTS AND METHODS

All patients had biopsy-proven Hodgkin's disease or non-Hodgkin's malignant lymphoma, and the pathologic diagnosis was confirmed by the national lymphoma panel. All patients had generalized disease (stage III or IV), had failed at least one previous chemotherapy trial with or without prior radiotherapy, and had at least one measurable area of malignant disease to serve as an indicator lesion. Patients who had received cytotoxic chemotherapy within 3 weeks (6 weeks for nitrosoureas), those who had active infection or impaired bone marrow, liver, or kidney function, and those who were completely bedridden (ECOG performance status of 4) were excluded from the trial.

Prior to randomization, patients were stratified according to histology, performance status, and leukemic status (> 5000 immature lymphoid cells/mm^3 of peripheral blood). Patients were then randomly assigned to receive ICRF-159 in either a loading course regimen (750 mg/m^2 in three divided doses daily for 3 consecutive days with courses repeated at 28-day intervals) or a weekly regimen (1.0 g/m^2 in three divided doses given 1 day each week).The drug was given orally in each regimen. Provisions were made for dose escalation if no myelosuppression or other toxic effects were observed, and doses were decreased on the basis of the degree of myelosuppression resulting from preceding courses. Patients had weekly wbc and platelet counts throughout the study.

Complete response was defined as the complete regression of all palpable and roentgenographically demonstrable disease. Partial response was defined as a > 50% reduction in the sum of the products of the dimensions of measurable lesions or at least a 30% decrease in the distance between the costal margin and the inferior border of the liver or spleen.

A total of 98 patients were entered in the study; 49 were randomized to receive treatment with each dose schedule. Ten patients were excluded from the loading course regimen and six patients were excluded from the weekly regimen due to ineligibility, failure to begin protocol therapy, gross violation of chemotherapy administration, or failure to submit acceptable case records. Thus, 39 patients receiving the loading course and 43 patients receiving the weekly treatments were evaluable.

Pretreatment characteristics of the 82 evaluable patients are summarized in table 1. The treatment groups were well-balanced with respect to all characteristics except prior weight loss. Significantly more patients with any degree of documented pretreatment weight loss were randomized to the loading course regimen.

RESULTS

Five of 39 patients (13%) treated with the loading course schedule and six of 43 (14%) treated with the weekly schedule experienced objective tumor responses (one complete and ten partial). Characteristics of the responding patients are shown in table 2. There was no difference in response rates between treatment regimens. Patients with diffuse non-Hodgkin's lymphomas had a lower response rate (7%) than those with Hodgkin's disease (15%) or nodular non-Hodgkin's lymphoma (24%). Likewise, patients with an ECOG performance status of 0 or 1 (ambulatory > 50% of the day) had a 17% objective response rate as opposed to a 9% objective response rate in patients with a performance status of 2 or 3 (ambulatory ≤ 50% of the day). Tumor responses were observed in 22% of patients with no weight loss in the 6 months prior to therapy but in only 5% of patients with any degree of recorded pretreatment weight loss. One patient receiving the loading course schedule and four patients receiving the weekly schedule had dose escalations, and of these only one (patient 11, table 2) experienced an objective tumor response.

Patient Survival

Survival curves were calculated using the Kaplan-Meier estimation technique and were compared using the log-rank statistic. Patients receiving the weekly treatment schedule had a significantly longer survival than patients receiving the loading course regimen ($P = 0.04$). The respective median survival times were 24 and 12 weeks. In addition, the following nontreatment variables were significantly associated with patient survival ($P \leq 0.01$): histology (patients with nodular lymphomas experienced longer survival than those with Hodgkin's disease or diffuse non-Hodgkin's lymphomas), performance status (ambulatory patients lived longer than nonambulatory patients), and weight loss prior to therapy (patients experiencing any pretreatment weight loss had shorter survival).

To examine whether the difference in patient survival between treatment regimens was due to im-

TABLE 1.—Pretreatment clinical characteristics

Characteristic	% of patients receiving— Loading course regimen (39 patients)	Weekly regimen (43 patients)
Age (yrs)		
≤ 40	23	30
> 40	77	70
Sex		
Male	69	63
Female	31	37
Histology		
Hodgkin's disease	18	14
Nodular non-Hodgkin's lymphoma	31	30
Diffuse non-Hodgkin's lymphoma	51	56
Performance status		
0–1	54	63
2–3	46	37
Weight loss in previous 6 mos		
No	36	61
Yes	64	39

TABLE 2.—Characteristics of responding patients

Patient No.	Age (yrs)	Sex	Histology*	Performance status†	Prior chemotherapy‡	Indicator lesion(s)	Response duration (wks)§
				Loading course regimen			
1	57	M	NPDL	1	BP; L; BCOP	Nodes, chest wall, lung	7
2	65	M	DH	2	BLEO-COP; ADR	Subcutaneous nodules	4
3	40	M	HD	0	MOPPr; CCNU-VPPr; ADR-BLEO-C	Liver	24
4	39	F	HD	3	BLEO-MOPPr	Nodes, subcutaneous nodules	14
5	35	M	DWDL	0	CP	Nodes	4
				Weekly regimen			
6	62	M	NPDL	1	UP; CVP; MOPPr; BLEO-ADR-P	Nodes	4
7	70	F	DPDL	2	MOPPr; BLEO-ADR-P; meCCNU-OC	Spleen, nodes	5
8	52	F	NPDL	0	COP; BLEO-P; ADR-COP	Abdominal mass	52+
9	67	M	DWDL	1	ADR; COP; L	Nodes	12
10	68	F	NPDL	2	CP; BLEO-COP; BCNU-ADR-0	Nodes	2
11	48	F	DWDL	0	L; COP; ADR-COP; BLEO-COP	Nodes	14

*NPDL = nodular poorly differentiated lymphocytic lymphoma; DH = diffuse histiocytic lymphoma; HD = Hodgkin's disease; DWDL = diffuse well-differentiated lymphocytic lymphoma; and DPDL = diffuse poorly differentiated lymphocytic lymphoma.

†ECOG scale: from 0 (fully ambulatory) to 4 (completely bedridden).

‡B = BCNU; P = prednisone; L = Leukeran (chlorambucil); O = Oncovin (vincristine); BLEO = bleomycin; C = cyclophosphamide; ADR = Adriamycin (doxorubicin); M = mechlorethamine; Pr = procarbazine; V = vinblastine; U = uracil mustard; meCCNU = methyl-CCNU.

§Measured from time remission was documented to date of relapse.

balances in prognostic factors (ie, histology, performance status, and pretreatment weight loss), these factors were simultaneously examined using a covariate survival model (4). This analysis indicated that patients receiving the weekly treatment regimen experienced longer survival than those receiving the loading course schedule ($P = 0.04$), independent of differences in histology, performance status, and weight loss between the two patient groups.

Toxicity

Toxicity was primarily hematologic. Nine of 39 patients treated with a loading course schedule had life-threatening leukopenia and/or thrombocytopenia (wbc count nadir $< 1000/mm^3$ or platelet count nadir $< 25,000/mm^3$) whereas only three of 43 patients receiving the weekly course regimen experienced similar degrees of myelosuppression ($P = 0.04$). Two of the patients receiving the loading course regimen died of complications of infection which were considered at least partially due to treatment. Overall, 38% of patients receiving the loading course regimen had a wbc count nadir $< 2000/mm^3$ and 26% had a platelet count nadir $< 50,000/mm^3$. Twenty-eight percent of patients receiving the weekly regimen had a wbc count nadir $< 2000/mm^3$ and 19% had a platelet count nadir $< 50,000/mm^3$. Patients with impaired performance status (nonambulatory $\geq 50\%$ of the day) had a significantly higher risk of experiencing severe myelosuppression as opposed to those with better performance scores ($P = 0.03$), regardless of treatment regimen. Prior radiotherapy was not associated with an increased incidence of severe myelosuppression among patients treated with the loading course schedule, although only one of 12 patients (8%) treated with the weekly schedule who did not have prior radiotherapy experienced a wbc count nadir $< 2000/mm^3$ and none had a platelet count nadir $< 50,000/mm^3$.

DISCUSSION

This study has confirmed a previous report of antitumor activity of ICRF-159 in patients with advanced malignant lymphomas (1). However, duration of response in our study, with either dose regimen, was substantially shorter than that reported by the WCSG. In the WCSG study, all patients received weekly treatment at a starting dose level identical to the weekly regimen in our investigation. Only one of our 11 responding patients had a response duration > 1 year, whereas five of the eight patients in the WCSG study had responses lasting > 1 year.

Although objective responses were observed with both treatment schedules, the weekly method of administration appears superior on the basis of fewer episodes of life-threatening hematologic toxicity and longer patient survival. Our data also indicate the importance of performance status, histology, and pretreatment weight loss as prognostic discriminants which should be taken into account in the design of future studies of new chemotherapeutic agents in patients with advanced refractory malignant lymphomas. The low response rate and increased risk of serious toxic effects among nonambulatory patients lead us to question the value of entering such patients in phase II studies of new cytotoxic agents.

Further study of ICRF-159 as a constituent in combination chemotherapy regimens for patients with less advanced malignant lymphomas appears warranted, although the moderate antitumor activity observed will have to be weighed against the considerable potential for myelosuppression and the availability of other new cytotoxic drugs for clinical trials.

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Phase II Study of L-Asparaginase in the Treatment of Pancreatic Carcinoma[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

L-Asparaginase was given to ten patients with advanced nonresectable pancreatic carcinoma because of the demonstration of in vitro sensitivity of the tumor to the drug. No therapeutic value was demonstrated for L-asparaginase. Toxicity was significant, mainly as evidenced by increasing mental confusion and early signs of a coagulopathy. On the basis of this limited study, L-asparaginase seems to have no value in advanced pancreatic carcinoma. The usefulness of L-asparaginase as primary therapy in patients with less advanced disease remains to be determined.

[Cancer Treat Rep 64:1359–1361, 1980]

L-Asparaginase, an enzyme commercially derived from *Escherichia coli*, has been used extensively as a therapeutic agent in human malignancies. Its antitumor effect is related to its ability to catalyze the hydrolysis of asparagine, an essential amino acid in some human tumors (1–3).

There is extensive literature on the clinical value of L-asparaginase (4–6), with most usefulness having been noted in acute lymphocytic leukemia where it is capable of producing a 50% rate of response (complete plus partial). L-Asparaginase has also been effective, though to a lesser degree, in acute myelocytic leukemia, the chronic leukemias, and the non-Hodgkin's lymphomas. Sporadically reported experiences in approximately 80 patients with a variety of solid tumors have not demonstrated significant tumor responses. We are unaware of any reported studies utilizing L-asparaginase in pancreatic carcinoma.

Our interest in this trial stemmed from the work of Wu et al (7). They cultured a cell line derived from a tumor of the tail and body of the pancreas of a 65-year-old white male (MIA PaCa-2) and tested the in vitro tumor-inhibitory effect of L-asparaginase on the cellular growth. L-Asparaginase in concentrations of 0.5–1.0 IU/ml was inhibitory and lethal in this and another (Panc. 1) pancreatic carcinoma cell line but not in human melanomas or breast cancer. Because of these in vitro effects on pancreatic carcinoma and the well-known clinically reported incidence of pancreatic toxicity with L-asparaginase (abdominal pain and tenderness, diarrhea, increasing amylase and glucose levels, and hypoinsulinemia), a clinical trial of the drug in pancreatic carcinoma was instituted.

METHODS

All patients with histologically proven, measurable adenocarcinoma of the pancreas with unresectable lesions were eligible for therapy. Prior therapy was allowable but all toxicity from such chemotherapy and/or radiotherapy had to have resolved before L-asparaginase therapy was begun.

Baseline studies included a medical history, physical examination, blood cell counts, serum chemistries, serum amylase, and CEA. Measurable disease was assessed either by physical examination, roentgenogram, or appropriate liver scans or pancreatic sonograms. Serial blood cell counts and chemistries were repeated weekly during treatment courses, and tumors were measured twice weekly during hospitalization and weekly thereafter. Where necessary, liver scans and pancreatic sonograms were repeated monthly.

The treatment protocol initially consisted of L-

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[2]This study was conducted by the Southeastern Cooperative Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: University of Miami School of Medicine and the Comprehensive Cancer Center for the State of Florida (*H. E. Lessner, S. Valenstein, R. Kaplan,* and *A. Yunis,* CA-05641) and the University of Kentucky, Lexington (*P. DeSimone,* CA-20255).

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TABLE 1.—Clinical characteristics of patients treated with L-asparaginase

Patient No.	Sex	Age (yrs)	Duration of disease (mos)	Previous treatment	Clinical symptoms
1	M	55	4	Radical pancreatoduodenectomy, 5-FU, methyl-CCNU, vincristine	Hepatomegaly, liver pain, ascites
2	M	72	3	None	Jaundice, weakness
3	F	81	6	Laparotomy	Jaundice, weight loss, anorexia
4	F	62	7	Laparotomy, 5-FU, methyl-CCNU	Severe abdominal pain, weight loss
5	F	65	4	Laparotomy, ICRF-159, radiation therapy	Abdominal pain, large mass, ascites
6	F	69	3	Laparotomy, bypass	Jaundice, weakness, pain
7	M	50	5	None	Abdominal pain, weakness, fever
8	M	58	5	5-FU, mitomycin C, streptozocin	Jaundice, abdominal mass, hepatomegaly
9	M	52	9	Laparotomy, methyl-CCNU, 5-FU, vincristine	Hepatomegaly, jaundice
10	M	53	2	Laparotomy	Hepatomegaly

TABLE 2.—L-Asparaginase treatment

Patient No.	No. of days of treatment	Total dose (IU)	Treatment-limiting factor
1	7	420,000	Disease death
2	8	360,000	None
3	9	405,000	Mental confusion
4	14	630,000	Mental confusion
5	5	270,000	Mental confusion
6	10	360,000	Mental confusion
7	4	200,000	Mental confusion
8	10	270,000	None
9	14	326,000	Hypoglycemia
10	14	224,000	Coagulopathy

asparaginase at a dose of 1000 IU/kg/day in a continuous iv drip for 14 days. Because of encephalopathy in the first three treated patients, the schedule was thereafter decreased to 1000 IU/kg/day for 2 days followed by 500 IU/kg/day for 8 days.

RESULTS

Table 1 summarizes the clinical data for the ten patients placed in this protocol. Five patients had had no prior chemotherapy whereas the other five patients had been treated with a variety of agents as noted. All patients had advanced, active disease.

Table 2 summarizes the L-asparaginase treatment delivered to this group of patients and the treatment-limiting factors. Treatment courses ranged from 4 to 14 days and were stopped because of increasing mental confusion, hypoglycemia, or a bleeding diathesis. No patient received a second course of therapy since none demonstrated a salutary effect from the first course and most had significant toxic reactions. Mental confusion occurred as early as Day 3 of therapy in one patient but generally was first noted at Days 7–10. Clearing of the confusion took 3–10 days after treatment was stopped, which is consistent with the half-life of L-asparaginase (approximately 15–20 hours) and the known cumulative effects of daily L-asparaginase administration. Other toxic reactions of significance included laboratory evidence of coagulopathy as measured by falling fibrinogen levels and rising prothrombin and partial thromboplastin times (four patients) and mild liver function abnormalities (two). At autopsy, one patient (No. 5) was noted to have hepatocellular degeneration consistent with toxic hepatitis of uncertain etiology. Another patient developed hypoglycemia. No objective or symptomatic responses were seen.

Serum L-asparaginase levels were studied in two patients, and the serum levels were well above the therapeutic levels suggested by the in vitro assay technique. Both patients attained serum levels of 10–30 IU/ml while the in vitro inhibitory levels were 0.5–1.0 IU/ml.

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Treatment of Advanced and Recurrent Squamous Cell Carcinoma of the Cervix With Combination Doxorubicin and Cyclophosphamide[1]

Brief Reports and Preliminary Communications

P. Hanjani* and S. Bonnell[2,3]

Doxorubicin used as a single agent and in combination with other drugs is one of the most highly active anticancer agents in a broad spectrum of solid tumors. In squamous cell carcinoma of the cervix, doxorubicin alone has produced reported response rates in the range of 10%–40% (1–3). When it was used in combination with other agents such as vincristine (4), methotrexate (5–7), cisplatin (8), and bleomycin (1), the results have not exceeded those of doxorubicin alone. Cyclophosphamide has also been used as an anticancer agent for squamous cell carcinoma of the cervix, with response rates in the range of 10%–20% (9,10). Laboratory studies have demonstrated that the combination of doxorubicin and cyclophosphamide exhibits significant therapeutic synergism. Also, in clinical use, this combination has been proven to be an effective therapy for advanced adenocarcinoma of the breast, ovary, and endometrium (11–14). We treated 20 patients with advanced cancer of the cervix with this combination.

MATERIALS AND METHODS

At the State University of New York, Upstate Medical Center, Section of Gynecology-Oncology, 20 patients with biopsy-proven recurrence or metastatic squamous cell carcinoma of the cervix not amenable to further surgery or radiation therapy were studied from January 1, 1975, through December 30, 1977. Prior therapy included radiation therapy alone or radiation therapy plus surgery (18 patients) and surgery alone (two). No patient had received prior chemotherapy. Each patient had a lesion which could be measured by pelvic examination or roentgenogram.

Patients received doxorubicin at a dose of 50 mg/m² and cyclophosphamide at a dose of 500 mg/m² on Day 1 repeated every 3–4 weeks as tolerated. Three patients who had had recent radiation therapy (Nos. 3, 12, and 16) initially received a decreased dose of doxorubicin (37.5 mg/m²) and cyclophosphamide (375 mg/m²) iv. If the nadir wbc count was ≥ 3000/mm³ and the nadir platelet count was ≥ 100,000/mm³, the following course was escalated to the full dose. The maximum dose of doxorubicin was 550 mg/m².

Patients were evaluated according to the following criteria: *complete response*—the complete disappearance of clinical evidence of disease and relevant symptoms for ≥ 3 months; *partial response*—a ≥ 50% reduction in the size of the lesion; *improvement*—a < 50% decrease in the size of the measurable tumor; *stationary disease*—no change in the size of the lesion; and *disease progression*—an increase in the size of the measurable tumor or the appearance of a new lesion. Criteria used to evaluate subjective response were the patients' perception of better quality of life as demonstrated by less pain, increase in appetite, weight gain, and decrease in lymphedema; patients were required to meet two of the criteria for ≥ 2 months to be considered as subjective responders. For the final results, only those patients with a complete or partial response were evaluated as responders. Patients were evaluated for response by a single examiner during the period of this study.

RESULTS

Sites of metastases and response data for the 20 patients are shown in table 1. Thirty percent of the patients had a partial or complete response, with a mean survival of 13.2 months. The mean progression-free interval for this group was 10 months. One patient (No. 14) had a complete response for > 20 months. The longest progression-free interval was experienced by patient 17 who had a > 75% reduction in the size of her pelvic mass for 21 months. Her death was not cancer-related. The remaining 70% of the patients who did not respond

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TABLE 1.—Summary of data for 20 patients

Patient No.	Sites of metastases	Response status	Duration of response (mos)	Survival (mos)
1	Pelvis	Progression	—	5
2	Vagina	Progression	—	4
3	Cervix	Stationary	3	4
4	Pelvis	Partial	2	5
5	Bone	Stationary*	9	12
6	Supraclavicular node	Stationary	5	5
7	Inguinal lymph node, lung	Stationary*	2	4
8	Inguinal node	Stationary	4	12
9	Vaginal vault	Progression	—	6
10	Pelvis	Stationary*	10	14
11	Inguinal lymph node, bone	Partial	2	9
12	Pelvis	Partial	4	5
13	Pelvis	Improved*	2	6
14	Cervix	Complete	20	27
15	Supraclavicular node, lung	Stationary	3	4
16	Pelvis	Progression	—	6
17	Cervix and vaginal lesion	Partial	21	21
18	Pelvis	Improved	5	7
19	Pelvis, vaginal vault	Partial	11	12
20	Cervix	Stationary*	8	12

*Subjective response.

had a mean survival of 7.2 months, with a mean progression-free interval of 3.6 months.

Five patients listed in table 1 (Nos. 5, 7, 10, 13, and 20) had a subjective response despite the fact that they did not respond clinically. Durations of response in these five patients were 8, 2, 6, 2, and 4 months, respectively.

Most patients experienced some toxic reaction to this chemotherapy regimen. Nausea, vomiting, and alopecia were universal but not dose-limiting. One patient (No. 13) had life-threatening leukopenia after four courses of chemotherapy, when her wbc count dropped to 200/mm^3. At that time she had progression of disease with brain metastases, and she died 6 months after diagnosis. Hemorrhagic cystitis developed in one patient (No. 14). Drug-induced cardiac toxic effects were not seen in any patient. No radiation recall was observed at radiated sites.

DISCUSSION

Our 30% response rate with doxorubicin and cyclophosphamide for advanced or recurrent squamous cell carcinoma of the cervix is slightly higher than that reported in similar studies; however, survival and duration of response are not significantly better, and the results may even be inferior to the response obtained with other combinations of drugs (15). In 1978, Alberts and Ignoffo (16) published their 20% partial-plus improved response rate. Dosage of doxorubicin and cyclophosphamide in their study was slightly lower than in ours. In a recent randomized study by the Gynecologic Oncology Group employing the combination of doxorubicin and cyclophosphamide as one of the treatment regimens, the overall response rates for pelvic recurrence and extra-pelvic metastases were 15.4% and 24%, respectively (4). Median survial of 6 months and median progression-free interval of 3.5 months for all 20 patients in our study are similar to their results (median survival of 7.3 months and median progression-free interval of 3.9 months) for patients receiving a doxorubicin and cyclophosphamide regimen.

In summary, the response rate of 30% and palliative role of the combination of doxorubicin and cyclophosphamide in the treatment of advanced or recurrent squamous cell carcinoma of the cervix in our study is not significantly different from that reported in other studies using this combination. Also, this result is not better than the results obtained from other drug combinations in the treatment of cervical neoplasms (15, 17, 18).

The results of these studies indicate that the combination of doxorubicin and cyclophosphamide does not improve the response rate over that of doxorubicin used alone (4) and hence the suggestion that cyclophosphamide and doxorubicin are synergistic in laboratory animals was not proved here. The combination of doxorubicin with other chemotherapeutic agents to treat this neoplasm may be worthy of further investigation.

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Doxorubicin, Cisplatin, and *Corynebacterium parvum* in Non-Small Cell Bronchogenic Carcinoma[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Forty previously untreated patients with stage III non-small cell bronchogenic carcinoma were treated with doxorubicin (Adriamycin) at a dose of 60 mg/m² iv on Day 1 and cisplatin (*cis*-diamminedichloroplatinum[II]) at a dose of 60–80 mg/m² iv on Day 1, every 4 weeks. Each patient was randomized to receive iv *Corynebacterium parvum* either on Day 1 or on Day 14 of each 4-week cycle. There were no complete responses and only seven of 40 (17.5%) patients achieved a partial response, with an overall median survival of 23 weeks. Those patients with stable disease survived longest. There was no difference in median survival among those patients receiving *C. parvum* on Day 1 (25 weeks) and among those receiving *C. parvum* on Day 14 (17 weeks).

[Cancer Treat Rep 64:1367–1369, 1980]

A new approach in the treatment of non-small cell carcinoma of the lung is based on our demonstration (2) that survival in patients receiving *Corynebacterium parvum* and chemotherapy is increased compared to the survival in patients receiving either methanol-extracted residue of bacillus Calmette-Guérin or no immunotherapy and the same chemotherapy. The reasons for this increased survival are presently unknown, and the first question we attempted to answer in dissecting this *C. parvum* effect concerned the interval between the *C. parvum* and the chemotherapy. Would *C. parvum* be more effective on Day 1 or on Day 14 of each chemotherapy cycle?

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MATERIALS AND METHODS

Forty consecutive patients with stage III (TNM classification) bronchogenic carcinoma were treated between April 1977 and June 1978. Informed consent was obtained. There were 23 males and 17 females, 38–78 years of age, with a median and mean age of 60 years. The ages of patients did not differ significantly between sexes. Performance status was evaluated according to Zubrod's modification of the Karnofsky scale (3). Initially, patients were randomized to one of three treatment arms; however, the second arm was discontinued early in the study. Patients in arms one and two received iv *C. parvum* on Day 1 while patients in arm three received *C. parvum* on Day 14 of each 4-week cycle. All patients were treated with doxorubicin (Adriamycin) at a dose of 60 mg/m² iv on Day 1 and cisplatin (*cis*-diamminedichloroplatinum[II]) at a dose of 60–80 mg/m² iv on Day 1, every 4 weeks. Arm two was discontinued after seven patients were entered; it was identical to arm one except for the addition of bleomycin at a dose of 15 mg/m² im on Day 1. Iv *C. parvum* (0.5–2 mg) was given according to patient tolerance, and was preceded by 100 mg of iv hydrocortisone. The criteria for complete response, partial response (PR), stable disease, and disease progression have been previously reported (2). Patients without a PR or disease progression who were not observed for two cycles of therapy (because of early death or discontinuation of chemotherapy) were considered evaluable but were not classified according to response category. Analyses were done using the life-table method (4) to present the data graphically. A multiple regression technique, Cox modeling (5), was also used to build a model to predict survival.

RESULTS

The 40 patients are listed according to treatment arm in table 1. Of 36 patients with measurable disease, five (19%) achieved a PR, 12 (33%) had stable disease, and six (17%) had rapidly progressing disease. Thirteen patients (36%) had responses which

TABLE 1.—Characteristics of the patients in each treatment arm

Characteristic	Treatment				
	1	2	3	1 + 2	Total
Total No. of patients	17	7	16	24	40
Males	11	6	6	17	23
Females	6	1	10	7	17
Median age in yrs	61	61	59	61	60
Median performance status	2.1	1.4	1.5	1.9	1.7
No. of patients with—					
Adenocarcinoma	7	1	7	8	15
Squamous cell carcinoma	7	3	5	10	15
Large cell carcinoma	3	3	4	6	10
PR	3		4	3	7
Progressive disease	3		1	3	4
Stable disease	3	3	6	6	12
Unclassified response	8	4	5	12	17
Median survival in wks	15	91*	17	25	23

*Misleading because 4 of 7 patients survived < 8 wks.

could not be classified because they had received only one cycle of chemotherapy without disease progression or PR. The number of patients with PRs and stable disease is nearly identical to that in our previous protocol (No. 960401) (stable disease, 18%; disease progression, 29%). The following variables were found by chi-square analysis to have no significant effect on response rate: age, sex, treatment arm, histology, and performance status.

Table 2 lists the variables analyzed for significant influence in survival. Patients receiving *C. parvum* on Day 1 (arms one and two) have a slightly longer but not significantly different median survival than patients receiving *C. parvum* on Day 14 (25 versus 17 weeks). In the analysis of survival by performance status (table 2), the better the performance status, the longer the median survival. When groups 1, 2, and 3 were combined, they were significantly different from group 4 ($P < 0.01$). There was a significant difference in response classification (table 2) between the progressive disease group and the combined groups of partial responders and those with stable disease ($P < 0.001$). Patients with stable disease had the longest median survival (58 weeks), which was statistically different ($P < 0.01$) from that of patients with PRs (29 weeks). Sex and histology had no significant effect on survival. No other significant differences between groups were observed. When results in patients (protocol 970501) are compared to the results of our previous protocol (No. 960401), the median survival times are the same (23 weeks).

TABLE 2.—Median survival and variables studied

Variable	Survival (wks)
Performance status	
1	44
2	35
3	28
4	10
1 + 2 + 3	34
Response category	
PR	29
Stable disease	58
Progressive disease	14
PR + stable disease	47

DISCUSSION

The purpose of this protocol was to compare the effect of *C. parvum* given on Day 1 versus Day 14. Patients receiving *C. parvum* on Day 1 survived longer but not significantly longer than those receiving *C. parvum* on Day 14. We are unable to explain the longer survival of the patients with stable disease when compared to those with PR. This has not occurred in previous studies (2,7). However, there is a murine model for this finding using *C. parvum* and chemotherapy (8). As in the past, survival in our study was calculated from Day 1 of chemotherapy and there were no significant time intervals between diagnosis and initiation of therapy that might interfere with the interpretation of the survival data. When the survival data are analyzed by performance status, results agree with the previously reported findings (2,5). The overall efficacy of this treatment does not appear to represent a significant advance when compared to other reported regimens (9); however, it is nearly identical to our previously reported study (2).

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Combination Chemotherapy for Advanced Cutaneous T-Cell Lymphomas[1,2]

Brief Reports and Preliminary Communications

Jahan Raafat, Martin W. Oster,* Paul Lo Gerfo, and Richard L. Edelson[3]

SUMMARY

Seven patients with advanced cutaneous T-cell lymphomas were treated with a combination chemotherapy regimen of vincristine, doxorubicin (Adriamycin), bleomycin, methotrexate, leucovorin factor, 5-FU, and hydrocortisone. All patients had an objective response but only one had a complete clinical remission. Our results are compared to other regimens reported in the literature.

[Cancer Treat Rep 64:1371–1374, 1980]

Among the lymphomas is a distinct subgroup characterized by a malignant proliferation of T cells that characteristically infiltrate the skin (1). Mycosis fungoides and Sézary syndrome are parts of the spectrum of these cutaneous T-cell lymphomas. Recent studies have demonstrated that these malignant T cells tend to have helper activity when cultivated with B cells (2).

Although initially often limited to the skin, the cutaneous T-cell lymphomas frequently disseminate to visceral organs, including the lymph nodes and blood. Therapy for early cutaneous-only stages tends to involve such dermatologic treatments as topical agents and electron-beam radiation therapy. The limited experience with combination chemotherapy in treating the disseminated stages has recently been reviewed (3). We wish to report our results using a combination chemotherapy regimen for solid tumors, which is well-tolerated and whose components are all active against lymphomas (4).

MATERIALS AND METHODS

Seven patients with advanced cutaneous T-cell lymphomas seen from 1976 to 1978 were entered in this pilot study (table 1). All seven patients had biopsy-proven lymph node as well as skin involvement with lymphoma. In addition, two patients had bone marrow involvement (one had hepatomegaly and the other had splenomegaly) and five had abnormal T cells in the peripheral blood. According to the classification system of Bunn and Lamberg (5), there were five patients with stage IVA and two with stage IVB disease. Prior therapy included nonspecific dermatologic creams (five patients), electron-beam radiation therapy (three), and oral steroids (two).

The treatment regimen was adapted from a combination chemotherapy regimen designed for squamous cell carcinomas of the head and neck and based on cytokinetic principles (4). The regimen consisted of monthly cycles of vincristine (2 mg by iv push) and doxorubicin (Adriamycin) (50 mg by iv push at Time 0), bleomycin (60 mg as an iv infusion from 12–18 hours), hydrocortisone (500 mg by iv push at 12 and 18 hours), methotrexate (100 mg by iv push at 12, 15, and 18 hours), 5-FU (500 mg by iv push at 18 hours), and leucovorin (15 mg im or orally every 6 hours for four doses starting at 26 hours) (fig 1).

RESULTS

The toxicity of this combination chemotherapy was minimal, with only occasional mild myelosuppression and stomatitis. There were no deaths, granulocytopenia-related infections, or thrombocytopenic bleeding episodes related to therapy. Only occasional dose adjustments were needed aside from a 50% dose reduction of all drugs in a frail 75-year-old woman.

Responses were seen in all seven patients. Lymph node involvement and response were assessed by

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TABLE 1.—Patient characteristics

Patient No.	Age, sex	Disease stage	Extracutaneous involvement	Prior treatment	No. of chemotherapy courses	Response*	Sites of response	Duration of response (mos)	Duration of survival from start of chemotherapy (mos)
1	71, M	$T_4N_3M_0$ (IVA)	Lymph nodes†	Topical	7	PR	Skin, lymph nodes	9	17
2	63, M	$T_2N_3M_0$ (IVA)	Lymph nodes†	Electron-beam radiation therapy, topical	8	CR	Skin, lymph nodes, blood	9	10
3	50, F	$T_3N_3M_0$ (IVA)	Lymph nodes,† subcutaneous nodules†	Topical, prednisone, electron-beam radiation therapy	5	PR	Skin, lymph nodes	5	7
4	43, M	$T_4N_3M_1$ (IVB)	Lymph nodes,† spleen, bone marrow†	—	7	PR	Skin, lymph nodes, spleen	10	17
5	60, F	$T_3N_3M_1$ (IVB)	Lymph nodes,† liver, bone marrow†	Topical, prednisone	4	PR	Skin, lymph nodes, liver, blood	3	5
6	70, M	$T_4N_3M_0$ (IVA)	Lymph nodes†	—	12	PR	Skin, lymph nodes, blood	14	22
7	75, F	$T_3N_3M_0$ (IVA)	Lymph nodes†	Topical, electron-beam radiation therapy	10	PR	Skin, lymph nodes	18	22+

*PR = partial response; CR = complete response.
†Biopsy-proven.

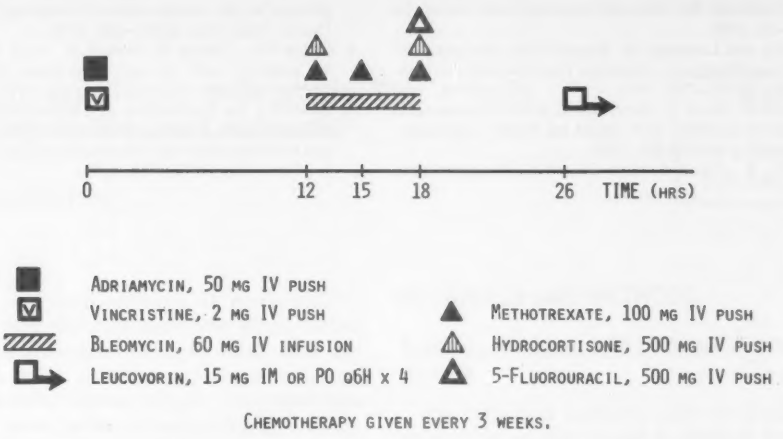

FIGURE 1.—Chemotherapy schedule for patients with advanced cutaneous T-cell lymphoma.

palpation and not by lymphangiography. One patient had a complete clinical remission of skin, lymph node, and peripheral blood involvement for 9 months while six patients had partial remissions (at least 50% reduction in lymph node and skin involvement). All patients had improvement or complete clearing of skin and lymph node involvement. While both patients with bone marrow involvement had improvement of peripheral blood smears, neither had clearing of bone marrow invasion. Spleen size returned to normal in the one patient with extensive splenomegaly, and liver size returned to normal in the one patient with hepatomegaly. Neither patient had had a liver or spleen tissue diagnosis made.

Mean remission duration was 9.7 months (range, 3–18). Six patients have died of progressive disease after partial remissions lasting 3–14 months. One patient is alive 22 months after the start of treatment, having achieved an 18-month partial remission.

DISCUSSION

The cutaneous T-cell lymphomas are a distinct subgroup of malignant lymphomas that have a predilection for skin involvement. Nevertheless, extracutaneous involvement is frequent, as has recently been demonstrated by using extensive staging and cytogenetics procedures (6). For that reason, systemic chemotherapy is often needed in the management of patients with such lymphomas. Unfortunately, only a limited experience with such systemic therapy has been reported. In a recent review, seven regimens used to treat a total of 55 patients were summarized (3). The largest number of patients treated by any particular regimen was only 12. The results of these studies were similar to ours in that (*a*) most patients (81%) achieved a response, (*b*) complete responses were uncommon (22%), and (*c*) most response durations were brief (< 1 year).

Since our results using a somewhat different and more complicated chemotherapy regimen appear similar but not superior to those of studies previously reported, we currently are using a more conventional regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone). We also wish to suggest the use of combined modality therapy, perhaps incorporating such newer approaches as the use of antithymocyte globulin (7). Preliminary reports from the University of Chicago (8) and the National Cancer Institute Veteran's Administration Medical Oncology Branch (9) on the use of combined modality therapy with electron-beam radiation and drug combinations have suggested superior results compared to those we achieved using combination chemotherapy alone.

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Doxorubicin and Ricin, a Strongly Synergistic Combination in Mouse Leukemia[1]

Brief Reports and Preliminary Communications

Ø. Fodstad* and A. Pihl[2]

SUMMARY

The effect of combinations of doxorubicin (Adriamycin) and the cancerostatic plant lectin ricin on L1210 leukemic cells in mice was assessed by end-point dilution and spleen-colony assays. The combinations, which were well-tolerated by the mice, acted synergistically on leukemic cells in liver, spleen, and brain. In the bone marrow, a dramatically synergistic effect was obtained with combinations that had only slight effect on the survival of normal stem cells.

[Cancer Treat Rep 64:1375–1378, 1980]

Major advances in cancer chemotherapy have been achieved by the concurrent or sequential administration of several different drugs (1–3). With the many clinically active cytostatic agents now available, the number of possible combinations is vast, and a systematic search for better drug combinations must therefore be made in animal models (4). In mice with L1210 leukemia, we have studied combinations of doxorubicin (Adriamycin), one of the most useful drugs in the treatment of various types of human cancer (5), and ricin, a toxic plant lectin that has previously been shown to have cancerostatic activity in certain murine tumors (6–8) as well as on a number of human cancers growing as xenografts in athymic, nude mice (8,9). In contrast to most cytostatic agents, ricin is a protein synthesis inhibitor (10,11) and its toxicity (12) is different from that of doxorubicin.

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MATERIALS AND METHODS

Tumors, Animals, and Chemotherapeutic Agents

The lymphoid leukemic cells were serially transplanted as ascitic tumor in DBA/2 mice. Mice of both sexes, weighing 20–25 g, were used. Doxorubicin was obtained from Farmitalia, Milan, Italy, and ricin was prepared from the seeds of *Ricinus communis* (13). The median lethal dose for doxorubicin was 18 mg/kg and the minimum lethal dose for ricin was 2.7 μg/kg.

End-Point Dilution and Spleen-Colony Assays

Mice were made leukemic by iv injection of 1×10^6 L1210 cells on Day 0, and the animals were divided into appropriate groups. One group was used as control, whereas the other groups were treated on Day 2 with the indicated doses of doxorubicin, ricin, or the two drugs given concurrently. The mice were killed on Day 5 and cell suspensions were prepared from the different tissues.

The end-point dilution assay was carried out essentially as described by Hewitt (14). Appropriate dilutions of cell suspensions obtained from bone marrow, liver, spleen, and brain from treated and untreated animals were injected ip into recipient mice and the survival time was recorded. The fraction of leukemic cells that survived treatment was estimated by determining the titer of the cell suspension from treated and untreated animals, resulting in the same mean lifespan of the recipient mice.

The spleen-colony assay for leukemic bone marrow colony-forming units (CFU) was performed as previously described (7). Appropriate dilutions of the bone marrow cells were injected iv into recipient mice; the spleens were removed and the macroscopic colonies formed were counted 8 days later. The average number of colonies obtained from the

drug-treated animals was expressed, relative to that obtained from the untreated control animals, as the fraction of surviving CFU per femur. To test the effect on normal bone marrow stem cells, non-leukemic mice were treated in the same way as the leukemic animals, but the bone marrow cells were injected into mice previously irradiated with 660 rads.

RESULTS AND DISCUSSION

The effect of each drug given individually has been reported previously (7). These results were confirmed here, as shown in figure 1. A moderate, selective effect of the individual drugs on leukemic bone marrow cells is demonstrated. The effect of increasing doses of doxorubicin given together with a fixed dose of 1 μg/kg of ricin on normal and leukemic bone marrow cells is shown in figure 2. The results observed are shown as fully drawn lines. It is apparent that for the leukemic cells the dose-response curve was linear in the semilogarithmic plot and that the spleen-colony and end-point dilution assays gave the same results. Clearly, the combinations used had a much greater effect on leukemic than on normal cells.

On the basis of the data obtained for the individual drugs, theoretic curves can readily be constructed for the effect expected if the two drugs acted additively. Such curves are shown as dashed lines in figure 2. In leukemic cells, the observed effect was much greater than that expected on the basis of additivity. In contrast, in normal bone marrow cells, no synergistic effect was found. In fact, the survival curve for the combination lies somewhat above that expected. Normal bone marrow cells that were induced to proliferate rapidly (15) were much less sensitive than the leukemic bone marrow cells (data not shown).

Administration of 1 μg/kg of ricin together with 7.5 mg/kg of doxorubicin reduced the fraction of surviving leukemic cells to about 1×10^{-6}, whereas the value expected if the two agents acted additively was about 1×10^{-1} (fig 2). To our knowledge, a synergism of this magnitude has not been previously reported for cytotoxic agents.

It should be noted that all of the combinations shown in figure 2 were well-tolerated by the mice. In separate toxicity experiments the LD50 for doxorubicin was found to be the same with or without the addition of 1 μg/kg of ricin, indicating that the overall toxicity of these two agents is not additive.

The effect of the combination on leukemic cells surviving in liver, spleen, and brain was determined by end-point dilution assay. Each experiment was repeated one or two times. The results are shown

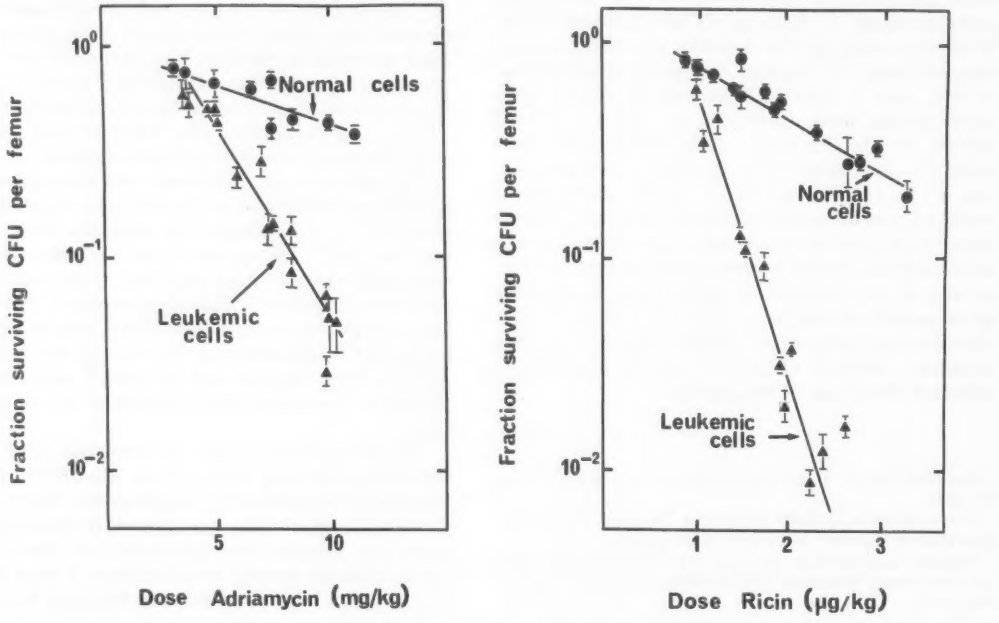

FIGURE 1.—Effect of doxorubicin (Adriamycin) (left panel) and ricin (right panel) on the survival of normal and leukemic bone marrow cells. Each point = mean ± SE for 8 mice in the spleen-colony assay.

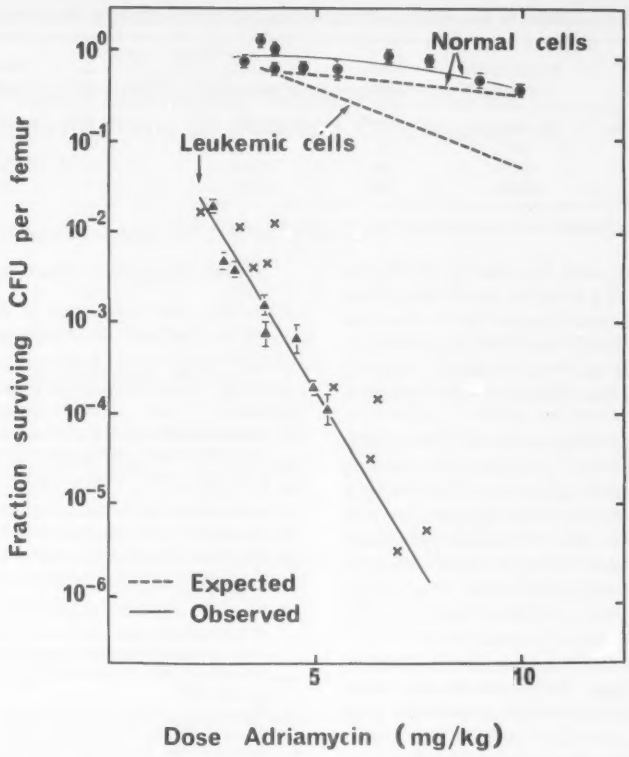

FIGURE 2.—Effect of the combination of doxorubicin (Adriamycin) and ricin on the survival of normal and leukemic bone marrow cells. —— = observed results, where $\overset{\top}{\bullet}$ and $\overset{\top}{\blacktriangle}$ = mean ± SE for 8 mice in the spleen-colony assays. X = values obtained by the end-point dilution assay for leukemic bone marrow cells. - - - - = expected values of the combinations, calculated from the effect of each drug given alone on the assumption that the 2 drugs acted additively.

in table 1 together with corresponding data for bone marrow. Ricin alone (1 μg/kg) had no effect on leukemic cells present in liver, spleen, and brain. Doxorubicin alone (5 mg/kg) had a definite effect that was most pronounced in the spleen. The combination reduced the percent of surviving leukemic cells in the tissues to much lower values than those expected on the basis of additive action. In some experiments, where the dose of doxorubicin in the combination was varied, the response was strongly correlated to the doxorubicin dose. However, whereas this dose-dependent variation in response was pronounced in the bone marrow (fig 2), only small differences were seen in the other tissues.

Although the synergistic effect was considerably less dramatic in liver, spleen, and brain than that observed in the bone marrow, the results indicate that the effect on the bone marrow cannot be accounted for by a redistribution of leukemic cells. The great difference in the degree of synergism in the tissues examined is not understood. Preliminary experiments suggest that it may be related to differences in drug access to the leukemic cells.

We then tested the effect of the combination on the survival of leukemic mice. When the mice were injected iv with 1×10^6 cells, the untreated animals in the control group had a mean lifespan of 5.1 days. In the other groups, the mice were treated on Day 3, ie, in a state of advanced leukemia. There were no long-time survivors in any of the groups. The administration of 1 μg/kg of ricin alone did not enhance the survival (5.0 days), whereas 10 mg/kg of doxorubicin alone increased the lifespan by 134% (12.0 days). Concurrent administration of the two drugs in the doses indicated gave an increased lifespan of 198% (15.2 days). Since it has been found that the generation time of L1210 cells injected iv is about 9.5 hours and that a tenfold reduction in

TABLE 1.—Effect of doxorubicin and ricin on leukemic cells in different mouse tissues

Tissue	Doxorubicin (5 mg/kg)	Ricin (1 μg/kg)	Doxorubicin + ricin		Expected/ observed
			Expected*	Observed	
Bone marrow	50	67	33.5	0.017	2000
Liver	10	100	10	2–5	2–5
Spleen	0.5–1	100	0.5–1	0.1–0.3	3–5
Brain	25–50	100	25–50	1–5	10–25

*Value calculated on the assumption that the 2 agents acted additively.

the number of leukemic cells injected iv resulted in an increased lifespan of 1.3 days (16), it can be estimated that the increased lifespan (here observed due to the addition of ricin) involved a reduction in the average number of surviving leukemic cells by 2–3 logs, compared to that obtained with doxorubicin alone.

The finding that the lectin ricin, in a dose that alone had little or no effect, is capable of strongly potentiating the antileukemic effect of doxorubicin, raises a number of questions. Experiments are now in progress to study whether a similar synergism is found in other forms of leukemia and with substances related to ricin and doxorubicin. The nature of the interaction may not be simple and requires further study.

In human leukemias the bone marrow is assumed to be the tissue of origin. Moreover, in the treatment of leukemias the toxic effect on normal bone marrow cells is frequently the dose-limiting factor. On this background, the synergistic effect of doxorubicin and ricin observed here on leukemic bone marrow cells in mice, together with the low toxicity of the combination on the normal bone marrow cells, may have interesting implications.

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Weekly *cis*-Diamminedichloroplatinum(II): Active Third-Line Chemotherapy in Ovarian Carcinoma—A Preliminary Report[1]

Brief Reports and Preliminary Communications

M. Steven Piver,* Shashikant Lele, and Joseph Barlow[2]

SUMMARY

Ten evaluable patients with progressive ovarian adenocarcinoma received third-line chemotherapy consisting of weekly cisplatin (*cis*-diamminedichloroplatinum[II]) at a dose of 1 mg/kg weekly for a maximum of 6 weeks followed by 60 mg/m² every 3 weeks. There was no life-threatening toxicity. Seven patients (70%) achieved an objective partial response. This is the highest response rate achieved, to date, by third-line chemotherapy in women with advanced ovarian adenocarcinoma. Weekly cisplatin therefore warrants a trial as first-line therapy.

[Cancer Treat Rep 64:1379–1382, 1980]

Until recently, there was no significantly effective second-line chemotherapy for patients with progressive ovarian adenocarcinoma. However, Vogl and co-authors (1) recently documented a 67% rate of response to second-line chemotherapy with hexamethylmelamine and cisplatin (*cis*-diamminedichloroplatinum[II]), with or without doxorubicin, in patients not responding to alkylating agents.

Because of the improved second-line responses, patients are now requiring third-line chemotherapy for progressive ovarian carcinoma after second-line chemotherapy. We have used cisplatin, primarily at a dose of 100 mg/m² every 3 weeks, as third-line chemotherapy in 20 such patients and achieved only one objective response (5%) (2).

Although we had not demonstrated cisplatin to be effective as third-line chemotherapy given every 3 weeks, it was decided to evaluate six weekly doses of cisplatin to be followed by cisplatin every 3 weeks. This was predicated on the successful use of such a regimen in nonseminomatous testicular tumors, seminoma, and prostate and bladder carcinoma (3). In July 1978, we admitted a patient with recurrent ovarian adenocarcinoma whose malignancy had progressed while she had been receiving 5-FU and cyclophosphamide and subsequently methotrexate and cyclophosphamide. In addition, she had a complete obstruction of the small intestine documented by small bowel roentgenograms. At the time of the exploratory laparotomy it was impossible to perform an intestinal bypass because of the extent of tumor involvement in the abdomen.

Due to moderate bone marrow depression, the patient received the planned six weekly courses over a 9-week period. Her intestinal obstruction was relieved as documented by roentgenographic evaluation and her ability to retain solid foods. Prior to weekly cisplatin therapy she was unable to retain even a liquid diet. A second patient with complete intestinal obstruction secondary to ovarian adenocarcinoma, not amenable to surgical correction, also responded to this regimen given as second-line therapy. The purpose of this report is to evaluate the response to weekly cisplatin as third-line chemotherapy in women with progressive ovarian adenocarcinoma.

MATERIALS AND METHODS

Twelve patients were entered in the protocol. Two of these patients were not evaluable, having died from their malignancy after the first week of cisplatin treatment. The ages of the ten evaluable patients ranged from 45 to 66 years. The histopathology of their cancers consisted of serous adenocarcinoma (five patients), clear cell carcinoma (two), poorly differentiated adenocarcinoma (two), and mucinous adenocarcinoma (one). First-line chemotherapy had consisted of high-dose methotrexate and cyclophosphamide (five patients), 5-FU and cyclophosphamide (four), and melphalan (one).

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Patients received a median of 8.5 courses/patient (range, one to 20). There were no objective responses; however, eight women had stationary disease prior to progression of their malignancy.

All ten patients were subsequently treated with second-line chemotherapy: methotrexate and cyclophosphamide (five) and cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin (CHAD) (five). They received a median of two courses (range, one to eight). There were no objective responses; however, four women had stationary disease prior to progression of their malignancy. All ten women then received weekly cisplatin as third-line chemotherapy within 6 weeks of progression of their malignancy while receiving second-line therapy.

A complete response was defined as the complete disappearance of all measurable disease lasting a minimum of 1 month. Partial response was defined as a $\geq$ 50% decrease in the product of the diameters of measurable tumor and/or complete regression of malignant effusions lasting for $\geq$ 1 month. Because of the unusual nature of this protocol, patients with massive disease resulting in complete intestinal obstruction were classified as partial responders if there was relief from tumor-caused intestinal obstruction, as documented by roentgenographic evaluation. Stationary disease was defined as absence of increase in measurable disease or malignant effusions. Progression was defined as a $\geq$ 25% increase in measurable disease and/or the appearance of new lesions.

Patients received cisplatin at a dose of 1 mg/kg/week. The plan was to administer this dose for 6 weeks and then to administer cisplatin at a dose of 60 mg/m^2 every 3 weeks. However, if the wbc count was $<$ 3000/mm^3 or the platelet count was $<$ 100,000/mm^3, cisplatin was delayed until the blood cell count improved. Lasix (40 mg) was given 1 hour before the administration of cisplatin. Cisplatin was added to 2000 ml of one-third normal saline and 5% dextrose and water with 18 g/liter of mannitol and infused over a 6-hour period. An additional 2000 ml of one-third normal saline and 5% dextrose was given over a 16-hour period. Prior to each course of chemotherapy, all patients had a physical examination, creatinine clearance, and SMA-12 including BUN and creatinine.

RESULTS

Seven (70%) of the ten patients had a partial response. This consisted of a $\geq$ 50% decrease in the size of pelvic masses (five patients), the complete disappearance of supraclavicular mass and a decrease in the pelvic mass (one), and the release of complete intestinal obstruction (one; patient 7) (table 1). Moreover, three of the five women who had received cisplatin in the CHAD combination chemotherapy subsequently responded to weekly cisplatin.

Durations of response were 12, 18, 20, 20, 26, 28, and 33 weeks, respectively, with two women continuing to respond at 20 and 28 weeks. Of the three patients who did not respond, one had progression of disease after the fifth week of cisplatin, one after the fifth course of weekly cisplatin given over 10 weeks, and one when she did not return for 3 weeks for her third weekly course of cisplatin (having been hospitalized elsewhere for upper respiratory infection).

Three of the patients were able to receive the planned six weekly courses of cisplatin therapy in 6 weeks. One patient was able to complete four weekly courses in 4 weeks and then refused weekly cisplatin but was agreeable to receiving therapy every 3 weeks. She is currently still responding at 28 weeks. One patient required 15 weeks to receive the six courses, one required 9 weeks, one required 10 weeks to receive five courses prior to disease progression, one had disease progression after five weekly courses of cisplatin, and one received four weekly courses in 7 weeks being unable to return because of difficulty in travel.

During the 50 weekly courses of cisplatin in the ten patients and the 27 courses given every 3 weeks to seven women, there were only two instances of abnormal creatinine clearance, both $<$ 40 ml/minute. In addition, there was no ototoxicity and no patient experienced life-threatening wbc counts ($<$ 1000/mm^3) or platelet counts ($<$ 50,000/mm^3). Only two patients had a wbc count nadir of $<$ 2000/mm^3, and only three had a platelet count nadir of $<$ 75,000/mm^3.

DISCUSSION

In an effort to find an effective third-line chemotherapy for women with progressive ovarian adenocarcinoma, we evaluated weekly cisplatin followed by cisplatin every 3 weeks in ten evaluable patients. Most patients had both extensive disease and extensive prior chemotherapy. There was no significant renal, otologic, or hematologic toxicity. Most importantly, 70% of the patients achieved an objective partial response lasting from 12 to 33 weeks, with two patients continuing to respond at 20 and 28 weeks, respectively. The mechanism of action of the response to 1 mg/kg of weekly cisplatin followed by cisplatin every 3 weeks, as compared to the lack of responses to cisplatin at a dose of 100 mg/m^2 every 3 weeks given as third-line chemotherapy, is

TABLE 1.—Patient response to prior treatment and third-line cisplatin therapy

Patient No.	First-line therapy			Second-line therapy			Third-line therapy				
	Regimen*	No. of courses	Response†	Regimen*	No. of courses	Response†	No. of weekly cisplatin courses/ No. of wks	No. of cisplatin courses given every 3 wks	Response†	Criteria	Duration of response (wks)
1	MECY	9	S → PD	CHAD	3	PD	6/6	2	P	Pelvic mass	12
2	Melphalan	20	S → PD	MECY	2	PD	6/15	4	P	Neck and pelvic masses	33
3	MECY	17	S → PD	CHAD	1	PD	4/7	2	P	Pelvic mass	26
4	FUCY	6	S → PD	MECY	5	S → PD	6/6	5	P	Ascites, pelvic mass	20+
5	FUCY	9	S → PD	MECY	2	S → PD	5/5	0	PD	Neck and abdominal masses	—
6	FUCY	8	S → PD	MECY	1	PD	6/9	3	P	Intestinal obstruction	20
7	MECY	2	PD	CHAD	6	S → PD	5/10	0	PD	Abdominal mass, ascites, pleural effusion	—
8	MECY	7	S → PD	CHAD	1	PD	2/5	0	PD	Abdominal and axillary masses	—
9	MECY	1	PD	CHAD	1	PD	6/6	4	P	Pelvic mass, pleural effusion	18
10	FUCY	15	S → PD	MECY	8	S → PD	4/4	7	P	Pelvic mass	28+

*MECY = Methotrexate + cyclophosphamide; and FUCY = 5-FU + cyclophosphamide.
†P = Partial response; PD = progressive disease; and S = stationary disease.

unclear. The rationale, however, was to use weekly cisplatin as an induction agent to be followed by cisplatin every 3 weeks as maintenance therapy. The lower dose would potentially be less toxic while the total cumulative dose during this induction period would be higher and possibly more tumoricidal. This altered frequency of drug administration may, in part, explain the effectiveness of this regimen. If so, it is of special significance since three of the five women who had not responded to monthly administration of cisplatin in the CHAD regimen responded to cisplatin on a weekly basis. Regardless of the exact mechanism of action, the unusually high objective rate of response to this regimen as third-line chemotherapy warrants its trial as induction chemotherapy in first-line treatment of women with advanced ovarian carcinoma.

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Phase II Evaluation of AMSA in Patients With Metastatic Lung Cancer[1,2]

Brief Reports and Preliminary Communications

W. C. Nichols,* R. T. Eagan, S. Frytak, J. N. Ingle, E. T. Creagan, and L. K. Kvols[3]

SUMMARY

Fifty-four patients with metastatic lung cancer were treated with AMSA. Patients with a performance score of 0–1, no prior chemotherapy, and normal liver function received a dose of 35 mg/m²/day iv for 3 days every 4 weeks; patients with a performance score of 2–3, any prior chemotherapy, or abnormal liver functions received a dose of 30 mg/m²/day on the same schedule. Among 53 evaluable patients, responses were noted in one with adenocarcinoma and in two with squamous cell carcinoma (overall response rate, 6%). We conclude that AMSA has limited activity in lung cancer when used in this schedule except perhaps in previously untreated squamous cell carcinoma. Only a limited number of patients with large cell or small cell carcinoma were treated.

[Cancer Treat Rep 64:1383–1385, 1980]

AMSA is one of a series of acridine derivatives synthesized by Cain and Atwell (1). Many of the acridine derivatives are active against L1210 and P388 leukemias (1,2); however, AMSA was singled out for clinical trial on the basis of superior activity against B16 melanoma (1). We report the results of a phase II evaluation of AMSA in patients with metastatic lung cancer based on results of reported phase I data using an intermittent 3-day schedule (3).

MATERIALS AND METHODS

Patients were considered eligible if they had histologic or cytologic evidence of metastatic squamous cell carcinoma (SQC), large cell carcinoma (LGC), small cell carcinoma (SMC), or adenocarcinoma (ACA) of the lung and had failed treatment with at least one prior chemotherapeutic regimen unless laboratory or clinical factors prevented these other treatment options. An area of measurable or evaluable tumor was required and patients had to complete at least one full 3-day course of treatment to be considered evaluable for response and survival. Contraindications to entry in the study included a wbc count < 4000/mm³, a platelet count < 100,000/mm³, a serum creatinine > 2.0 mg/100 ml, any elevation of direct or indirect bilirubin, or an Eastern Cooperative Oncology Group performance score (PS) of 4.

Fifty-four patients were entered in the study; 53 were considered evaluable. One patient received treatment on only 1 of the 3 days and was hospitalized with deteriorating respiratory status, preventing completion of the initial course of treatment. Twenty-one patients (40%) had received no prior chemotherapy. Thirty-five percent of the patients had a PS of 0–1, and 65% had a PS of 2–3. The pretreatment characteristics of patients are shown in table 1. Before initiation of treatment and prior to each subsequent treatment, patients were evaluated with a history, a physical examination, a record of specific tumor measurements, and a laboratory assessment of at least a cbc, a 12-panel chemistry group, a posteroanterior and lateral chest roentgenogram, and a fractionated alkaline phosphatase. Interim myelosuppression was assessed by weekly wbc and platelet counts.

AMSA was reconstituted in a solution of dimethyl acetamide and lactic acid and diluted to 500 ml of 5% dextrose in water. It was infused iv over 60 minutes daily for 3 days repeated every 3–4 weeks until the first evidence of progression. The starting dose depended on a patient's PS, prior treatment, and liver function. Patients with a PS of 0–1, no prior chemotherapy, and a liver alkaline phosphatase less than twice normal received 35 mg/m²/day

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TABLE 1.—Pretreatment characteristics of 54 patients treated with AMSA

Characteristic	Tumor type			
	ACA	LGC	SQC	SMC
No prior chemotherapy				
No. of evaluable patients	8	4	9	—
Median age in yrs (range)	66 (38–79)	52 (47–71)	66 (58–79)	—
Men/women	8/0	4/0	6/3	—
No. of patients with—				
PS				
0–1	1/3	0/2	0/1	—
2–3	2/2	1/1	3/5	—
Indicator lesion				
Measurable/evaluable	4/4	1/3	2/7	—
Prior chemotherapy				
No. of evaluable patients	15*	3	7	8
Median age in yrs (range)	55 (33–70)	57 (50–68)	61 (51–75)	61 (50–72)
Men/women	6/9	3/0	7/0	7/1
No. of patients with—				
PS				
0–1	1/4	1/1	0/2	0/3
2–3	7/3	1/0	2/3	1/4
Indicator lesion				
Measurable/evaluable	5/10	2/1	1/6	5/3

*1 patient with a mixed ACA/SQC, predominantly ACA.

for 3 days; patients with a PS of 0–1, liver alkaline phosphatase less than twice normal, but prior chemotherapy received 30 mg/m²/day for 3 days; and patients with a PS of 2–3, regardless of prior treatment or liver function, received 25 mg/m²/day for 3 days. After the first 12 patients were entered in the study, essentially no acute or chronic toxic effects were observed at the 25-mg/m²/day dose level so that subsequent patients with a PS of 2–3 or abnormal liver functions were initially treated at 30 mg/m²/day.

Patients with clearly measurable indicator lesions were evaluated using standard criteria for complete regression (CR), partial regression (PR), stable disease, and disease progression. Patients with evaluable but not clearly measurable disease were evaluated using our previously reported criterion (4). In general, two investigators had to agree that the indicator lesion(s) was unequivocally smaller (regression), unchanged (stable), or larger (progression). Twenty patients (37%) had measurable disease and 34 patients (63%) had evaluable disease. Patients who died or who failed to return for treatment were considered to have had tumor progression on the day of death or the day of scheduled return, whichever occurred earlier.

RESULTS

One tumor regression (4%) (a measurable PR) was noted among 23 patients with ACA. The responding patient had previously received a combination of VP-16-213, cyclophosphamide, doxorubicin, and cisplatin. Two responses (one PR and one evaluable regression [13%]) were noted among 15 patients with SQC, neither of whom had had prior exposure to chemotherapy. No responses occurred in eight patients with SMC, all of whom had had prior chemotherapy exposure. No responses occurred in seven patients with LGC, three of whom had had prior chemotherapy exposure. Twenty-six of the 53 evaluable patients (49%) had tumor progression after the first course of AMSA and therefore received only one course of treatment. Overall, three responses were noted in 53 patients (overall response rate, 6%). The response durations calculated from the first day of treatment were 113, 125, and 229+ days.

At present, 29 of the 53 patients have died. The median survival for all patients estimated from a Kaplan-Meier survival curve was 133 days (range, 15–237+). The estimated median survival times of patients with ACA, SQC, LGC, and SMC were 139, 189, 86, and 111 days, respectively.

Toxicity

A total of 104 separate 3-day courses were evaluable to assess acute toxicity. Overall, the drug

administration was well-tolerated, with only minor nausea, vomiting, or diarrhea. Two patients experienced acute erythematous reactions at the site of iv administration. One reaction was urticarial in nature, but mild, and responded to treatment with an antihistamine. One patient with a history of paroxysmal supraventricular tachycardia developed transient episodes of tachycardia immediately following courses of AMSA in the 35–40-mg/m²/day range. AMSA was discontinued even though the patient's disease was objectively stable after two cycles of treatment.

Very little interval toxicity (ie toxicity noted from Day 4 of a treatment cycle until the next scheduled treatment) occurred. No episodes of prolonged nausea or vomiting occurred. One patient developed stomatitis and one related moderately severe muscle cramps to AMSA treatment. Myelosuppression generally was mild, with severe cytopenia developing in only two of 40 evaluable initial courses of treatment. One patient developed prolonged thrombocytopenia (platelet count of 8000–33,000) following a course of palliative thoracic spine radiotherapy given 14 days after initiation of AMSA. The other case of significant myelosuppression (nadir wbc count of 700/mm³ and platelet count of 72,000/mm³) occurred in a patient who received a dose of 35 mg/m²/day despite having previously been treated with chemotherapy. Overall, of 40 evaluable initial cycles of treatment, a nadir wbc count < 4100/mm³ (median, 2900/mm³; range, 700–4100/mm³) was observed in 21, and a nadir platelet count of 150,000/mm³ (median, 72,000/mm³; range, 8000–137,000/mm³) was observed in only seven.

DISCUSSION

A phase II evaluation of AMSA was performed in patients with advanced lung cancer. Thirty-two of the 53 evaluable patients had prior cytotoxic treatment and the majority had a PS of 2 or 3. The overall response rate was 6%, with regressions noted in SQC and ACA but not in LGC or SMC. Thirteen patients received more than two cycles of treatment but no evidence of cumulative or chronic toxicity was noted in those patients. Used at the defined levels of 30 mg/m²/day for 3 days for patients with a poor PS or those previously treated and at 35 mg/m²/day for 3 days for patients with a PS of 0–1 and no prior treatment, AMSA is well-tolerated with only mild gastrointestinal toxicity and acceptable myelosuppression. There was a suggestion that AMSA may have increased cardiac irritability but this occurred in only one patient and was not life-threatening.

We conclude that AMSA has limited activity in lung cancer at the dose levels and schedules we employed; however, in this study only a limited number of patients with LGC and SMC were treated. Most activity occurred in previously untreated SQC (two of nine patients). Further evaluation of different schedules or higher dose levels for the 3-day schedule, particularly in SQC, are probably warranted.

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Phase II Trial of Baker's Antifol in Metastatic Renal Cell Carcinoma: A Southwest Oncology Group Study[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Fifteen patients with metastatic renal cell carcinoma were treated with Baker's antifol at a dose schedule of 250 mg/m² iv for 3 days every 3 weeks. One objective response was seen, and toxicity was minimal. No usefulness of Baker's antifol in the treatment of metastatic renal cell carcinoma was demonstrated.

[Cancer Treat Rep 64:1387–1388, 1980]

Baker's antifol (BAF) is a triazine folate antagonist which is like methotrexate in its inhibition of dihydrofolate reductase but slightly different from methotrexate in its mode of action (1). A phase I study (2) with this compound indicated that the major toxic effects included myelosuppression, dermatitis, stomatitis, and nausea. A dose of 100–150 mg/m²/day for 5 days repeated every 2 weeks was recommended. In a phase II study (3), the schedule was modified and patients received 250 mg/m²/day for 3 days; in the presence of abnormal liver function, the dose was reduced to 150 mg/m². A complete and partial response rate of 10% was seen in the phase II study among 138 patients with various malignancies; three responses among six patients with renal cell carcinoma were reported. In view of this report, further determination of the activity of BAF in this neoplasm appeared to be indicated.

MATERIALS AND METHODS

Patients with metastatic renal cell carcinoma not amenable to surgical therapy were eligible for the study. Patients may have received prior chemotherapy and were required to have adequate renal function (defined as a BUN < 30 mg/100 ml and a creatinine < 1.5 mg/100 ml) and adequate liver function (defined as a serum bilirubin < 1.5 mg/100 ml or hepatic enzyme [SGOT or alkaline phosphatase] elevation of less than twice the institutional normal). Patients were divided into good- and poor-risk categories. Poor-risk patients were defined as those having abnormal liver functions with a serum bilirubin between 1.0 and 1.5 mg/100 ml and a serum enzyme not elevated more than twice the institutional normal.

The starting dose of BAF for good-risk patients was 250 mg/m² administered as an iv infusion over 1 hour daily for 3 days. For poor-risk patients, the starting dose was 100 mg/m². Subsequent courses of therapy were given at 3-week intervals if no significant toxicity occurred, if the absolute granulocyte count was > 2000/mm³, and if the platelet count was > 100,000/mm³. The dose was escalated on the basis of granulocyte and platelet count nadirs: doses were increased by one level if no significant myelosuppression (absolute granulocyte count < 1000/mm³ or platelet count < 50,000/mm³) was noted. The dose levels (mg/m²) employed were as follows: 100, 150, 200, 250, 300, 350, and 400.

Cbcs with differential and platelet counts were done at weekly intervals. Liver function (including SGOT, SGPT, alkaline phosphatase, LDH, and bilirubin) and renal function (including BUN, creatinine, and urinalysis) were checked at 3-week intervals. Tumor measurements were made prior to each course of therapy, and radiographic studies were repeated as indicated. An adequate trial of therapy consisted of completion of one course of treatment. A complete remission was defined as the complete disappearance of all clinical evidence of tumor for a minimum of 4 weeks. A partial remission was defined as a > 50% decrease in the sum of the products of all diameters of measurable lesions or a

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> 30% reduction in the sum of measurements below the costal margin in patients with liver metastases, with no simultaneous increase in any lesion or appearance of new lesions. Stable disease was defined as a steady state or a response less than a partial remission for ≥ 2 months. Disease progression was defined as an increase of at least 50% in the size of any measurable lesion or the appearance of new lesions. Patients who responded to BAF or whose disease was stable continued to receive BAF until disease progression or relapse was observed.

RESULTS

Seventeen patients with metastatic renal cell carcinoma were entered in this study between March 1978 and January 1979. One patient died of rapidly progressing disease and was considered an "early death"; a second patient never received BAF. Fifteen patients were therefore evaluable for toxicity and response.

The 15 patients (12 males and three females) ranged in age between 34 and 68 years (median, 52). All patients had metastatic lesions, and sites of measurable lesions were as follows: lung (11 patients), abdomen (two), and liver and soft tissue (one each). Ten patients had received prior tamoxifen (five) and chemotherapy (bleomycin [one], CCNU [three], and vinblastine [one]). The initial dose level of BAF was 250 mg/m² in 12 patients and 100 mg/m² in three. The median number of courses of chemotherapy administered was 2.5 (range, one to 12).

One of 15 patients (7%) achieved an objective response; the disease site in the responder was soft tissue, and response duration was 30 days. Four of 15 patients (28%) had stable disease (median duration, 3 months; range, 2–5). Toxicity was moderate. Granulocytopenia (absolute granulocyte count, 400/mm³) occurred in one patient with abnormal liver function and hepatic metastases. Thrombocytopenia (platelet count < 100,000/mm³) was not observed, and the median platelet count nadir was 309,000/mm³ during the first course of therapy. The median number of days to platelet count nadir was 16. Other toxic effects included nausea and vomiting (46%), diarrhea (13%), mucositis (7%), dermatitis (13%), and transient elevations of BUN (26%). The dermatitis observed was a maculopapular erythematous rash that was self-limiting.

DISCUSSION

The results of this study indicate minimal usefulness of BAF in the treatment of metastatic renal cell carcinoma. The previously noted 50% response rate in a small number of patients could not be confirmed in this series. Only mild to moderate toxic effects were encountered, and the absence of significant myelosuppression was probably secondary to the fact that patients with severe liver disease and/or renal function abnormalities were excluded. In conclusion, no activity in renal cell carcinoma was demonstrated for BAF at the dose levels and schedule used in this study.

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An Abbreviated Phase II Trial of Thalicarpine[1]

Joseph T. Leimert,* Michael P. Corder, Thomas E. Elliott, and Jan M. Lovett[2]

SUMMARY

Thalicarpine, a plant alkaloid of novel structure, was evaluated in a phase II clinical trial. Fourteen previously treated patients with advanced malignant disease were given thalicarpine at a dose of 1100 mg/m² weekly as a constant 2-hour iv infusion. Common toxic effects included nausea, ECG changes, arm pain, and lethargy; less frequent effects included vomiting, tachycardia, hypotension, pain distant from infusion site, urticaria, chills, diarrhea, and mydriasis. There was no hematologic, hepatic, or renal toxicity. There were no complete or partial objective responses. Although the drug's true response rate in any given tumor type cannot be determined, its absence of activity in man, to date, and the recent closing of its IND, make further clinical investigation with thalicarpine unlikely.

[Cancer Treat Rep 64:1389–1390, 1980]

The utility of the vinca alkaloids in cancer treatment raises the possibility that other plant alkaloids may also have clinically useful antitumor activity. Among such agents is thalicarpine, an aporphine benzylisoquinoline alkaloid, originally isolated from the roots of *Thalictrum dasycarpum* (1). This dimeric alkaloid is structurally dissimilar to the alkaloids in current clinical use. In preclinical studies, thalicarpine and related compounds have shown antitumor activity against the Walker 256 carcinosarcoma[3] and against a monolayer culture of KB cells (2). Its physiologic effects in the rhesus monkey include depression of mean arterial and coronary perfusion pressures and increase in heart rate and carotid artery blood flow (3). Acute toxic effects in animals included convulsions, apnea, hypotension, cardiac arrest, salivation, lacrimation, conjunctivitis, and mydriasis. Chronic toxicity was renal and hepatic (4).

In a phase I study of thalicarpine in man, the maximally tolerated dose, which was associated with considerable drowsiness, was found to be 1400 mg/m² (5). Other acute toxic effects included arm pain during the infusion, mild nausea and vomiting, mild lacrimation, stuffy nose, facial flushing, hypertension, hypotension, and ECG changes. No persistent or delayed toxic effects were observed. Specifically, there was no hematologic, renal, or hepatic toxicity. The recommended dose from this trial was 1100 mg/m² given as a 2-hour weekly infusion. The weekly schedule also finds support in the long retention of thalicarpine after iv administration in man (6).

No antitumor effect was observed in the initial clinical trial of thalicarpine. However, its novel structure, the suggestion of antitumor activity in preclinical experiments, and its absence of hematologic toxicity provided the impetus for a phase II evaluation of thalicarpine.

PATIENTS AND METHODS

Fourteen patients with advanced malignant disease were admitted to this trial between June 1976 and October 1979. All patients had had pathologically confirmed diagnoses, had received extensive prior treatment, had manifested disease progression despite conventional therapy, and had measurable disease. Diagnoses included malignant melanoma (five patients), metastatic breast cancer (four), and diffuse histiocytic lymphoma, fibrosarcoma, hepatoma, Hodgkin's disease, and multiple myeloma (one each). Patients with cardiovascular disease were excluded. The administration of central nervous system depressants 4 hours before or after infusion of thalicarpine was prohibited.

Thalicarpine was supplied in 100-mg ampules by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Each ampule was diluted in 9.8 ml of sterile water for injection and added to 1 liter of 5% dextrose solution.

[1]Received Feb 15, 1980; revised Apr 7, 1980; accepted Apr 17, 1980.

[2]Department of Internal Medicine, College of Medicine, University of Iowa, Iowa City.

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Treatment was given as a constant iv infusion of thalicarpine at a dose of 1100 mg/m² into an arm vein for ≥ 2 hours, as tolerated, with monitoring of blood pressure and ECG changes.

RESULTS

Fourteen patients received one to eight infusions of thalicarpine (median, three). All patients are considered evaluable for response. Although one patient described decreased pain and narcotic requirement in the 2 weeks after thalicarpine was initiated, there were no complete or partial objective responses. Two patients refused further therapy after one and three infusions of thalicarpine, respectively. Neither patient had evidence of change in measurable disease parameter in the week following the last treatment. Patient 1, with metastatic melanoma, showed no change in a pulmonary lesion after eight infusions of thalicarpine. Disease progression in all other patients while they were receiving treatment required discontinuation of therapy.

Toxic effects included nausea (seven patients), vomiting (three), cardiovascular effects (two), ECG changes (eight), pain in the arm receiving the infusion (seven), and lethargy during and after the infusion (nine). Cardiovascular effects included tachycardia to 120/minute during an infusion in one patient and transient hypotension to 82/46 in another. ECG changes did not persist beyond the end of the thalicarpine infusion. In one patient, one infusion was terminated early due to multifocal premature ventricular contractions. Cardiovascular complications were not more common in patients who had previously been treated with doxorubicin.

During two infusions in two patients, however, treatment was discontinued prematurely due to arm pain. At the end of her first infusion, one patient experienced severe pain in her neck and shoulders which was relieved by meperidine; the pain recurred to a lesser degree after the second infusion. Two patients developed transient urticaria at the injection site. One infusion in one patient was terminated early due to shaking chills, and one patient experienced mild diarrhea following therapy. Three patients were noted to have mydriasis during treatment. No hematologic, hepatic, or renal toxicity was observed.

DISCUSSION

Our experience with thalicarpine has been similar to that reported by Creaven et al (5) in a phase I investigation. While toxicity was acceptable at the dose and schedule used, none of our patients, nor those previously reported, showed an objective response. The transient subjective improvement described by one of our patients is not sufficient evidence for thalicarpine activity.

The true response rate to thalicarpine for any given tumor type cannot be reliably estimated due to small patient numbers. However, the experience to date has generated little enthusiasm for continued clinical investigation with the drug. The Division of Cancer Treatment Decision Network Review of August 1, 1979, resulted in the closing of the existing IND for thalicarpine (7). It is unlikely that thalicarpine or its related plant alkaloids will be further investigated in clinical oncology.

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LETTERS

Long-Term Survival in Patients With Advanced Adenocarcinoma of the Pancreas

A 50-year-old white male was examined in June 1976 and was found to have extensive carcinoma in the body of the pancreas, with fixation to the aorta. Metastases were noted throughout the peritoneal cavity, omentum, and both lobes of the liver. The liver biopsy revealed a moderately differentiated adenocarcinoma (fig 1). The patient began treatment according to Eastern Cooperative Oncology Group protocol 5274; he received chemotherapy with 5-FU at a dose of 450 mg/m² for 5 consecutive days every 5 weeks through December 1977, when, because of a recurrence of abdominal pain radiating to the back, a computerized axial tomographic (CAT) scan was performed. The scan revealed numerous, large, rounded shadows of lesser density within the liver and a mass involving the head of the pancreas, the porta hepatis, and the posterior margin of the antrum of the stomach. The patient was then crossed over to chemotherapy with melphalan at a dose of 3.5 mg/m² orally, twice daily for 5 consecutive days every 5 weeks. In February 1978, the patient was removed from the study because of gastrointestinal bleeding and a palpable mass in the right upper quadrant. After the bleeding subsided, the patient began to receive chemotherapy with streptozocin at a dose of 500 mg/m² iv for 5 consecutive days every 5 weeks. A repeat CAT scan performed in November 1978 was remarkable in that there was a substantial decrease in the size of the head of the pancreas and a definable acinar pattern (fig 2). The large metastatic deposits previously seen in the liver had changed dramatically. There were a number of small lucent areas seen on the precontrast study, all of which disappeared on the postcontrast examination. The assumption that these represented vascular metastatic foci which became isodense on the postcontrast examination could not be excluded. A third CAT scan performed in June 1979 showed no significant change.

The patient remains alive and in good health as of April 1980; the hemogram is completely normal. Elevation of alkaline phosphatase to 120 IU (normal range, 20–115) remains the only abnormality. This patient survives 3¾ years from the date of diagnosis and has demonstrated an unequivocal response to chemotherapy with streptozocin.

We have used streptozocin alone or in combination to treat a total of six patients with adenocar-

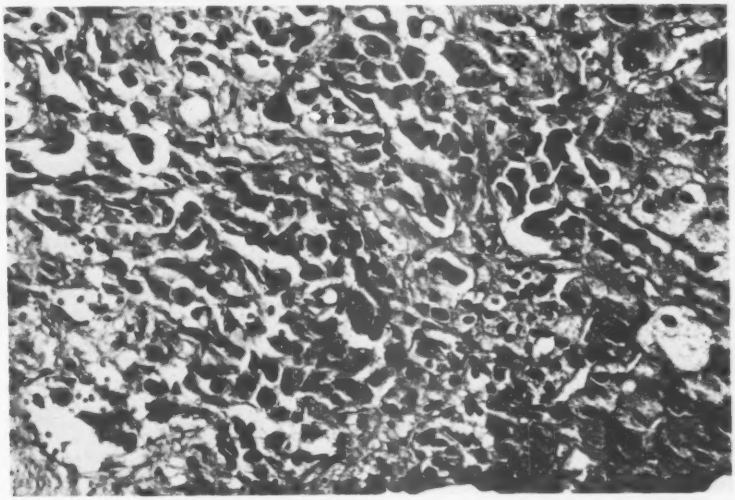

FIGURE 1.—Photomicrograph of liver biopsy specimen demonstrating metastatic adenocarcinoma of the pancreas (H and E stain × 440).

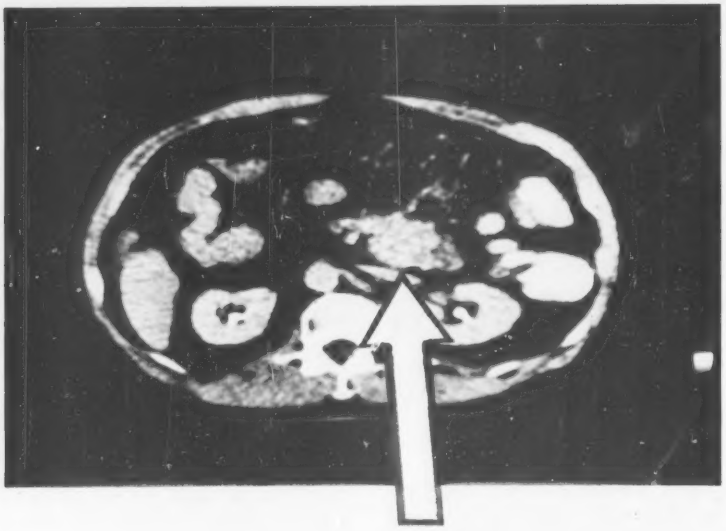

FIGURE 2.—Head of the pancreas and definable acinar pattern.

cinoma of the pancreas. The median survival of five patients is > 13 months. One patient was lost to followup. The median survival of the responders (three of six patients) is 20.6+ months.

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Severe Skin Necrosis Produced by Piperazinedione Extravasation

Skin and soft tissue necroses following paravenous extravasation of certain drugs have all too often been observed with commonly used antineoplastic agents such as doxorubicin, mechlorethamine, and vincristine. These agents are known for their potential in this regard. The necrosis generally results from the corrosive action of the agent on local tissues. These reactions may be severe and lead to surgical interventions such as extensive debridement or amputation. In this report, we describe a case of severe skin necrosis due to the subcutaneous extravasation of an experimental antineoplastic agent, piperazinedione.

CASE REPORT

A 59-year-old man with glioblastoma multiforme had partial resection of the tumor which resulted in stabilization of his hemiparesis and resolution of his headaches. Unequivocal signs and symptoms of progressive disease (including increased tumor as observed on computerized tomographic [CT] brain scan) were evident 3 months later. After treatment with steroids, residual tumor was surgically resected and he received 6000 rads of whole-brain irradiation postoperatively. Examination at the completion of radiotherapy revealed persistent sensory deficits with improvement of lethargy and weakness. A CT brain scan documented decreasing brain edema and tumor. Chemotherapy was then started with piperazinedione at a dose of 12 mg/m^2 given by rapid iv. The patient received six, single iv dose cycles every 3 weeks without toxic effects and with clinical stabilization. On the seventh course, a subcutaneous infiltration of piperazinedione occurred at the iv site in the left antecubital fossa. Five days later, the arm became edematous and tender, and an area of skin necrosis was evident. Eight days later, the eschar sloughed. In spite of vigorous topical therapy with betadine and peroxide, the necrotic area enlarged. Four weeks after the infiltration, the necrotic area required repeated operative debridement. Because of fever and positive wound cultures for *Staphylococcus aureus*, the

FIGURE 1.—Skin of left antecubital fossa, 8 wks after piperazinedione extravasation.

patient was treated iv with Nafcillin. Eight weeks later, intraoperative debridement revealed deep necrosis down to the level of the brachial artery and vein extending into the bicep and tricep muscles (fig 1). Repeat CT scan of the brain showed no change in the size of the parietal tumor since his radiation therapy 6 months earlier.

Ten weeks after the drug infiltration, because of continued local necrosis, the left arm was amputated above the elbow. Gradual postoperative healing ensued; however, 10 days following the procedure the stump appeared dusky with a purulent discharge flowing from the closure site. Forequarter disarticulation was advised, but the patient died of massive upper gastrointestinal bleeding prior to any further surgical procedure. The family denied permission for autopsy.

COMMENT

Piperazinedione is an antitumor antibiotic extracted from the culture broths of *Streptomyces grisealateus* and has recently been tested in phase II trials for activity in a variety of malignant diseases (1–4). Some antitumor activity has been reported in lymphomas (3). Reported toxic effects in human trials have included myelosuppression (moderate to severe leukopenia and thrombocytopenia), alopecia, nausea, vomiting, stomatitis, rash, and fever (1–6).

In this report, we document piperazinedione as yet another agent causing severe soft tissue necrosis following paravenous extravasation. The necrosis in this patient was so severe that intraoperative debridement and, ultimately, amputation became necessary. The early stage began insidiously with a small amount of blistering and superficial loss of skin. A slow progressive necrosis of the skin then developed over a 1-month period leading to deep painful ulcers with exposure and necrosis of important deep structures. The ulcer base consisted of yellow, shaggy debris and, in spite of vigorous local debridement, it was difficult to find viable normal or granulation tissue. Further loss of normal tissue

was accelerated by local *Staphylococcus aureus* infection. The continued necrosis of deep tissue with involvement of major vascular structures, lack of viable tissue, development of fever, and signs of septicemia made amputation of the involved extremity necessary. Histologically, tissue from the ulcer showed necrosis, chronic inflammation, and fibrosis.

The proper emergency management of paravenous extravasation of necrotizing drugs is not well-defined. Local measures which have been used for doxorubicin-induced skin lesions include infiltration with bicarbonate and dexamethasone (7), steroids alone (8), and ice (8). Although clinical responses with local care are reported (7,8), the size and degree of necrosis in experimental animals have not been modified by any local measures (9). Further studies are needed to define specific early effective therapy for this problem.

For more advanced lesions, debridement, skin flaps, and skin grafting may be necessary. Such measures have been successful in doxorubicin-induced ulcers. The importance of wide local excision and grafting at the erythematous stage rather than at the necrotic stage has been emphasized (10–12). Careful observation for local infection and institution of appropriate antibiotic therapy, when necessary, may improve the chances of successful surgical therapy. If all else fails, as in this case, amputation of the involved extremity may be necessary.

Clearly, prevention is the easiest "therapy." Careful iv needle or catheter insertion with assurance of blood return prior to injection, slow push of the drug over 2–5 minutes in a rapidly running iv line, and avoidance of small-caliber veins (particularly over joint surfaces) are mandatory considerations when using parenteral drugs that have the potential for soft tissue necrosis when extravasated.

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Eleventh Annual Neurosurgery Postgraduate Course and Fifth Annual Neurosurgical Nurses' Conference

San Francisco, California, May 21–23, 1981

The Eleventh Annual Neurosurgery Postgraduate Course and the Fifth Annual Neurosurgical Nurses' Conference will be held at the San Francisco Hilton Hotel, California, on May 21–23, 1981. This course will provide an update on selected topics in clinical neurosurgery by a distinguished faculty. Pain, intracranial surgery, pediatric neurosurgery, and craniocerebral trauma will be discussed in depth. Current concepts will be stressed. In addition to didactic sessions, there will also be luncheon discussions between small groups of registrants and individual members of the faculty on a variety of common neurosurgical problems. Participants can expect to acquire information that will improve their diagnostic and therapeutic skills.

Guest faculty will include: Drs. Derek Bruce, J. William Fielding, E. Bruce Hendrick, Surl Nielsen, D. Gordin Potts, Robert Rand, Joseph Ransohoff, and Joel Seres.

This course is presented by the Department of Neurosurgery of the University of California School of Medicine in San Francisco, and it is sponsored by Extended Programs in Medical Education. The program is approved for AMA/CMA Category I credit and for nursing continuing education credit toward relicensure. *For further information, please contact*: Extended Programs in Medical Education, Rm 569-U, University of California, San Francisco, CA 94143 (415-666-4251).

Second Annual Meeting of the American College of Clinical Pharmacy

Scottsdale, Arizona, June 26–28, 1981

The Second Annual Meeting of the American College of Clinical Pharmacy will be held on June 26–28, 1981, at the Camelback Inn in Scottsdale, Arizona. The deadline for submitting abstracts on pharmacotherapeutics and pharmacokinetics is March 1, 1981. *Abstract forms are available from*: Secretary, 2411 Holmes St, Kansas City, MO 64108.

Third European Organization for Research on Treatment of Cancer-National Cancer Institute Symposium on New Drugs in Cancer Therapy

Brussels, Belgium, October 15–17, 1981

The Third European Organization for Research on Treatment of Cancer-National Cancer Institute (EORTC-NCI) Symposium on New Drugs in Cancer Therapy is scheduled for October 15–17, 1981, at the Institut Jules Bordet, Brussels, Belgium. The symposium will focus on phase I and phase II clinical data including drug pharmacology with investigational anticancer agents. Preclinical aspects in the new drug development area will also be covered with particular emphasis on the role of the human stem cell assay in the screening of new antitumor drugs. The program will include invited presentations and free communications. Free communications will be selected on the basis of submitted abstracts. Abstracts must be received at the EORTC Data Center by *August 31, 1981*. Seating is limited and preregistration is recommended. The registration fee is $135.00. *For further information, contact either*: Dr. M. Rozencweig or Dr. M. Staquet, EORTC Data Center, Institut Jules Bordet, 1, rue Héger-Bordet, B-1000 Brussels, Belgium; telephone: (2) 538.57.90, telex: 22773.

Cancer 1981/Cancer 2001—An International Colloquium

Houston, Texas, November 10–14, 1981

Cancer 1981/Cancer 2001–An International Colloquium will be held on November 10–14, 1981, at the Shamrock Hilton Hotel, Houston, Texas. This Colloquium will be held in lieu of the University of Texas M. D. Anderson Annual Clinical Conference. On the 10th anniversary of the National Cancer Program, outstanding authorities will take a hard look at the directions cancer research and treatment should take in the near future. Presentations will focus on promising avenues of cancer research and likely developments in related fields, such as imaging, artificial intelligence, and monitoring. Participants will consider the possible impact of likely developments on cancer patient care and the probable status of cancer patient care in the year 2001. Presentations will be made by both basic and clinical scientists.

***For additional information, contact*: Dr. C. Stratton Hill, Jr, Conference Coordinator, Rm 115, University of Texas M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston TX 77030 (713-792-2222).**

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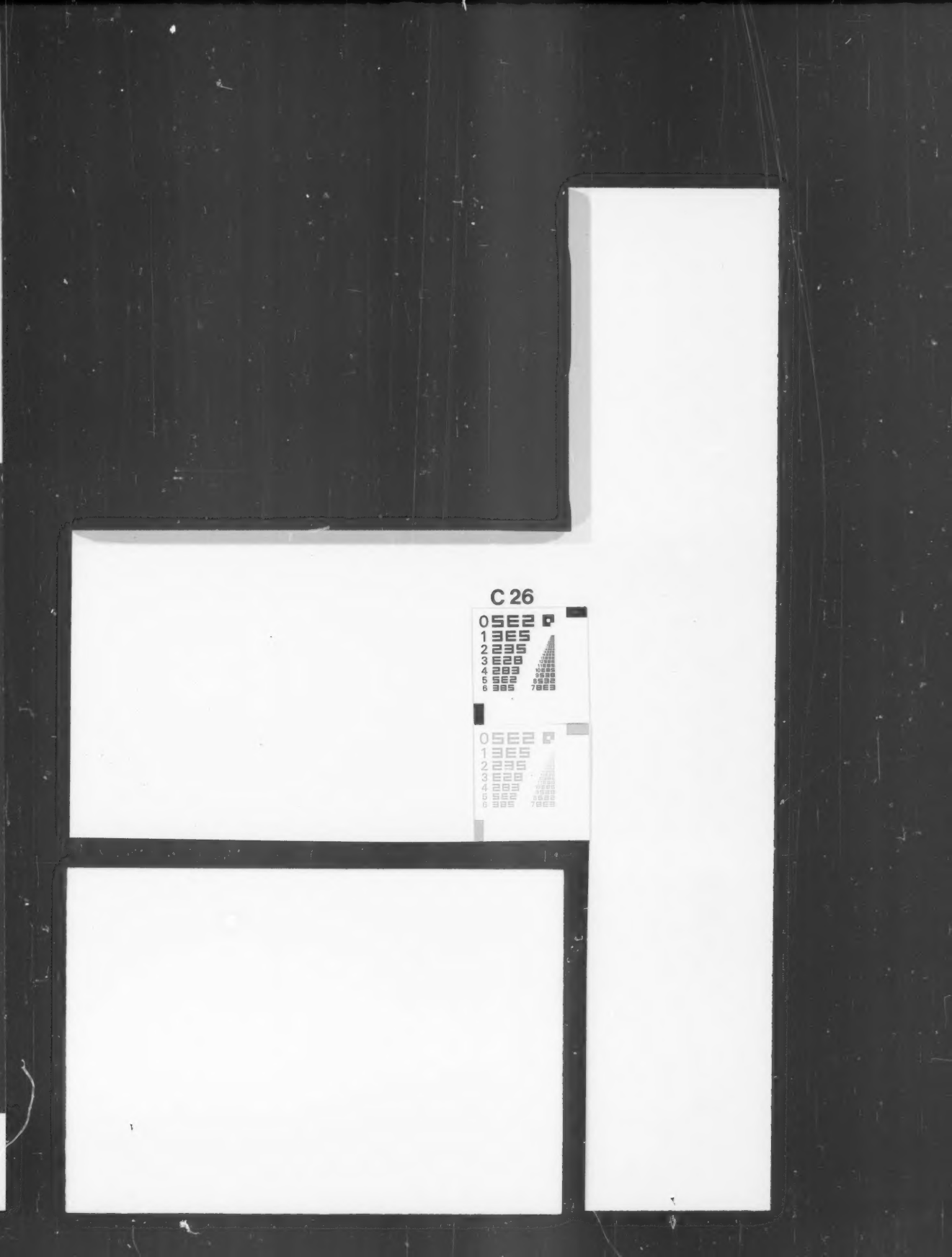
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